Polyamide microcapsules as a basis for the treatment of glaucoma.

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POLYAMIDE MICROCAPSULES AS A BASIS FOR THE TREATMENT OF GLAUCOMA

THESIS

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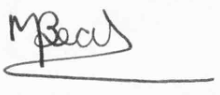
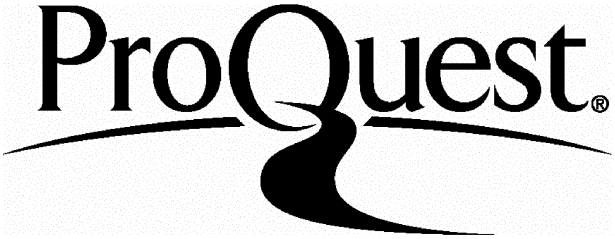
This research has been carried out in the School of Pharmacy and Pharmacology, under the supervision of N.E. Richardson, B.Pharm., Ph.D., M.P.S., D.A. Norton, B.Sc., F.P.S., F.I.Biol., D.B.A., A.C.T.(Birm.) and D.J.G. Davies, B.Pharm., Ph.D., F.P.S.





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SUMMARY

This work involves an investigation into the feasibility of using polyamide microcapsules as a basis for the treatment of glaucoma.

The Introduction considers conventional treatment of glaucoma together with novel ophthalmic drug delivery systems. Various methods by which microcapsules may be prepared are discussed and the preparation of polyamide microcapsules is discussed in detail. The properties of polyamides are described together with the properties of polyamide microcapsules. Consideration is also given to the theoretical release from microcapsules.

The Materials and Methods section lists the materials and instrumentation used throughout this work and describes the general methods used.

The Experimental section describes the development and preparation of nylon 6.10 and polyphthalamide microcapsules. This is followed by details of their properties including appearance, size distribution and release of encapsulated pilocarpine nitrate. Methods by which the preparation conditions were then modified are considered. These involve the addition of gelatin to the core, the use of short chain crosslinking molecules and the preparation of double walled microcapsules. The properties of these modified microcapsules are described. This section is followed by details of the structure of the microcapsule walls together with measurements of the permeability of polyphthalamide films. The final section of the experimental work discusses *in vivo* studies concerning the effect of microencapsulated pilocarpine nitrate on the rabbit eye and the dwell time of polyphthalamide microcapsules in the eye.

The final section of this thesis deals with the discussion of the experimental results obtained. The main conclusion drawn is that the walls of the polyphthalamide microcapsules do not significantly control the release of pilocarpine nitrate from the microcapsule core. This may arise due to the presence of pores in the microcapsule walls. Attempts to modify or reduce the release rate characteristics of the microcapsules by modification of the core and wall were unsuccessful.

CONTENTS

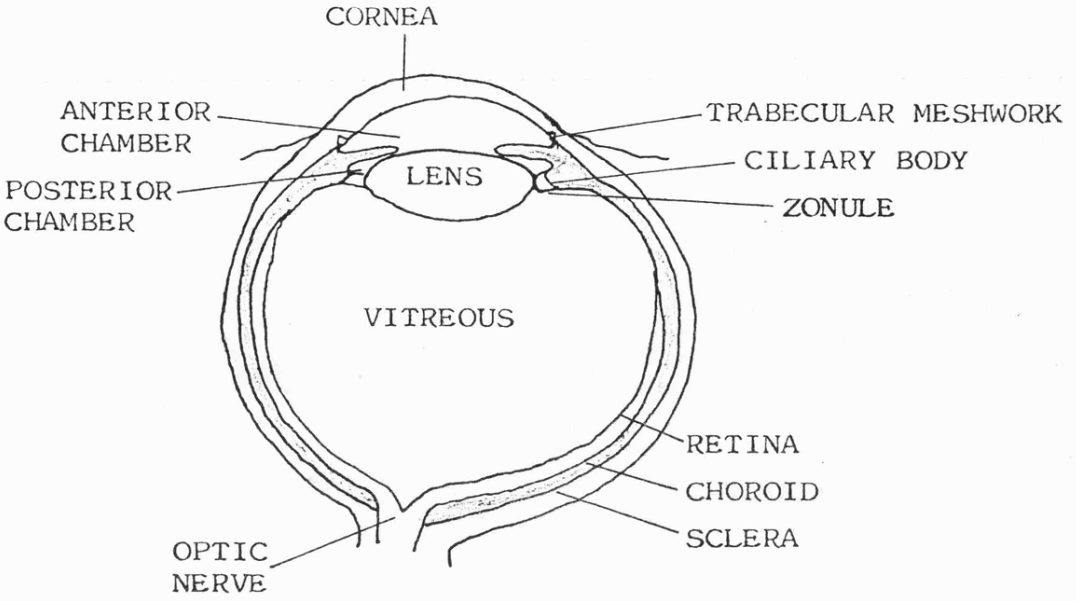
Page No.

INTRODUCTION

1.1 Glaucoma

1.1.1 Introduction

The adult human eyeball is a nearly spherical structure of approximately one inch diameter. It consists essentially of three separate concentric layers that are modified anteriorly to admit and dominate the passage of light. The outermost of these layers, the sclera, is purely protective; the innermost, the retina, is a light-sensitive recorder of images; and the intervening uveal layer contains numerous blood vessels. The anterior rim of this uveal layer is continued forwards to contain the intraocular muscles that govern the accommodative and pupillary movements, thus forming the ciliary body and the iris. The crystalline lens is attached to the ciliary body by means of the suspensory ligament or zonule. The cornea is divided into three layers: the epithelium, stroma and endothelium, each acting as a barrier to drug permeability. The epithelium and endothelium are rich in lipids and are more readily traversed by non-polar compounds; they are the principal barrier to ions and lipid insoluble materials. The stroma, richer in water content is readily penetrated by water-soluble polar compounds, but less so by non-polar compounds. The space between the cornea and the anterior lens surface is termed the anterior chamber, whereas the narrow circular space between the iris, the lens and the ciliary body is called the posterior chamber. Both anterior and posterior chambers contain aqueous humour, a thin watery fluid. The remainder of the eyeball is filled with the vitreous body which is a clear gel-like substance.



ANATOMY OF THE EYE

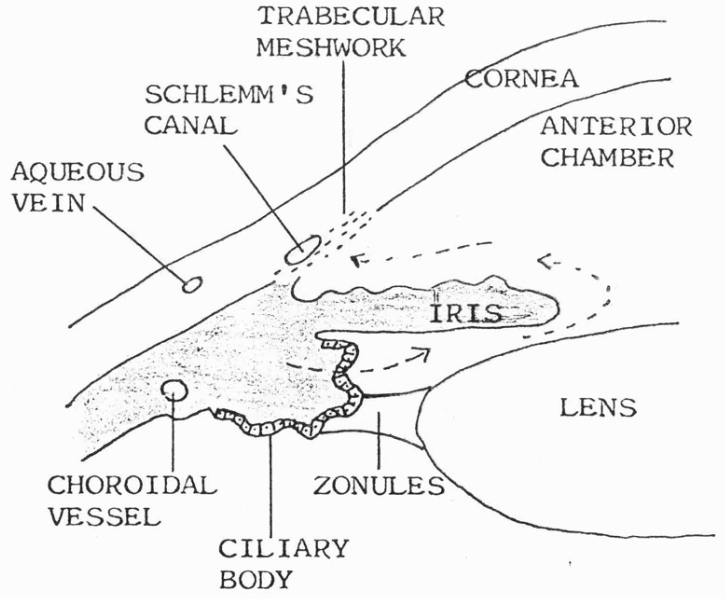


FIGURE 1.1 THE PRODUCTION AND OUTFLOW OF AQUEOUS HUMOUR.

The integrity of the shape of the eye is maintained by the internal positive pressure of the aqueous humour. This is produced by a combined process of secretion and diffusion which takes place throughout the blood-aqueous barriers of the eye. It is probable that most of the aqueous humour is formed by secretion and diffusion from the capillaries of the ciliary processes. The fluid is secreted into the eye with sufficient potential to produce an intraocular pressure of about 100mm Hg. However, the pressure usually maintained is in the order of 15 to 19mm Hg (1). Once formed, the aqueous flows into the anterior chamber via the pupil and then, largely under the influence of hydrostatic pressure, through the trabecular tissue in the angle of the anterior chamber and into the circular canal of Schlemm. From the canal it passes by small channels into the deep scleral capillary plexuses.

The condition of glaucoma arises as a result of an imbalance between the production of aqueous humour and its outflow through the canal of Schlemm. The most important characteristic of this condition is persistent or repeated elevation of intraocular pressure. This raised intraocular pressure eventually causes certain ophthalmological changes in the affected eye and if it is sufficiently high and persistent it may lead to irreversible blindness. The condition, which has been estimated to affect approximately 1% of the population over the age of 40 (2 - 5) may be divided into three types as shown below.

1.1.2 Classification of Glaucoma

1.1.2.1 Primary Glaucoma

This category is further subdivided into two types. The difference between the two is physical and refers to the angle tended by the cornea and the iris.

a) Closed Angle Glaucoma. Closed angle glaucoma is characterised by periodic bouts of raised intraocular pressure. The most prominent symptoms are pain and impairment of vision, caused by distension of the intra ocular tissues and waterlogging of the cornea. This condition is normally precipitated in narrow-angled eyes by a swelling or a forward displacement of the iris root. As a result of this swelling or forward displacement, the root of the iris may come into contact with the corneal epithelium. If this zone of contact becomes too extensive aqueous cannot escape and a hypertensive crisis ensues.

b) Open Angle Glaucoma. Open angle glaucoma is characterised clinically by a consistently raised intra ocular pressure, cupping of the optic disc and loss of visual field. Unlike closed angle glaucoma there is no associated pain. The exact aetiology of the disease is unknown but it is perhaps due to the factors listed below which may lead to an imbalance between the production and the outflow of aqueous humour.

1. Abnormal changes in the tissue of the filtration angle which gradually impede the passage of the aqueous humour.
2. Failure of action of the cells in the trabecular meshwork.
3. Sclerotic changes in the fine vessels which drain the aqueous humour from the canal of Schlemm.

4. An anomaly of the sympathetic or parasympathetic nerve complexes of the region.

1.1.2.2 Secondary Glaucoma

Secondary glaucoma arises as a result of a pre-existing pathological condition such as intraocular inflammation, lens dislocation, lens swelling and uveitis. It may also arise as a result of the metabolic upset following a central venous thrombosis. The symptoms are usually those of primary closed angle glaucoma but pain may be slight.

1.1.2.3 Congenital Glaucoma

This condition is due to the obstruction of the drainage of the aqueous humour by some form of congenital anomaly, for example malformation of the fibres of the pectinate ligament or a persistence of foetal tissue which obstructs the filtration angle. The increased intraocular pressure causes the infant globe to enlarge and the eyeball thus appears prominent. The measured intraocular pressure may be only slightly increased since this is largely mitigated by the distensibility of the globe. The optic disc is usually cupped and these eyes are normally myopic owing to the increase in their axial length.

1.1.3 Treatment of Glaucoma

1.1.3.1 Introduction

Treatment depends upon the type of glaucoma exhibited and upon the relative increase in the intraocular pressure of a particular patient. In closed angle glaucoma the first necessity is to constrict the pupil and draw the iris away from the angle of the anterior chamber. To this end pilocarpine is used, especially in combination with eserine. This régime may also be supplemented by systemic acetazolamide. The mode of action of the drugs is discussed in Section 1.1.3.2. Surgical treatment however is normally indicated in established closed angle glauxoma. The classical operation is a 'glaucoma iridectomy'. In this a wide sector of the iris is excised so that, over this area, the drainage angle can no longer be obstructed by the iris root.

The treatment of primary open angle glaucoma normally involves the use of drugs for example pilocarpine, adrenaline, guanethidine and timoptol. Where the intraocular pressure is not relieved by such treatment it may be necessary to establish a fistula through which the aqueous under tension can filter away.

In secondary glaucoma treatment of the underlying cause is called for, but when this is difficult, a reduction of aqueous production by acetazolamide is especially helpful. In cases of uveitis steroids can be used to diminish the exudate.

In congenital glaucoma only surgical treatment will avail and this is most simply effected by division of the pectinate ligament.

1.1.3.2 Mode of Action of Drugs used in the Treatment of Glaucoma

a) Parasympathomimetic Agents. The parasympathomimetic agents fall into two classes.

1. Cholinergic, for example pilocarpine, carbachol. (pilocarpine will be discussed in greater detail elsewhere)
2. Anticholinesterases, for example eserine, neostigmine, echothiopate.

They are all miotics and have the ability to reduce resistance to outflow of intraocular fluids. The site of action of the cholinergic drugs is at the parasympathomimetic receptors. The cholinergic activity of the anticholinesterases is achieved indirectly by inhibition of the enzyme acetylcholinesterase, thereby allowing increased amounts of acetylcholine to act for larger periods. The main disadvantage of treatment by miotics is they often produce ciliary spasm. The majority of miotics have activity of short duration and frequent instillations are necessary to control the intraocular pressure.

b) Sympathomimetic Agents. The sympathomimetic agents, for example, laevo adrenaline and phenylephrine are mydriatics and have the ability to reduce the formation of intraocular fluids. Their mode of action is stimulation of the sympathetic nervous system.

c) Sympatholytic Agents. These agents fall into two classes, adrenergic neurone blocking drugs and β receptor blocking drugs. The former block the release of noradrenaline and also cause some depletion of the noradrenaline stores at the adrenergic nerves. The principal drug in this class, guanethidine,

reduces the intra ocular pressure by decreasing the production of aqueous humour. The β receptor blocking drugs, for example, timoptol also decreases the production of aqueous humour by blocking the actions of the catecholamines.

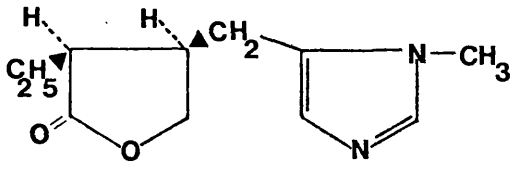
d) Diuretic Agents. Carbonic anhydrase inhibitors, that is diuretic agents, (for example acetazolamide) have a general effect on metabolism resulting in an increase in loss of water accompanied by an increase in the excretion of sodium and potassium. A result of the diuresis caused is a lowering of the intra ocular pressure.

e) Osmotic Agents. The osmotic agents raise the osmolarity of the plasma so that the fluid is drawn out of the eye with a resultant fall in ocular tension. Their effect is only temporary, becoming ineffective as soon as osmotic equilibrium is re-established. The drugs used in this type of treatment are urea, mannitol and glycerol.

In the case of diagnosed open angle glaucoma miotic treatment at the earliest stages in both eyes is essential. For this purpose pilocarpine 1 - 2% is the drug of choice (6).

1.1.3.3 Pilocarpine

Pilocarpine is a parasympathomimetic alkaloid.



It has the muscarinic actions of acetylcholine but is resistant to the hydrolytic action of the cholinesterases and thus has a useful duration of effect. It is stable, penetrates the cornea, is well tolerated and rarely toxic. The isomer of pilocarpine, isopilocarpine, has little or no therapeutic effect.

When instilled into the eye pilocarpine causes pupillary constriction, stimulates the ciliary muscle so as to increase accommodation and increases the permeability of the trabecular meshwork. The mechanism by which this increase in permeability occurs is not fully understood but may be due to the increase in prominence of cellular bulges in the endothelial monolayer of the trabecular wall of the canal of Schlemm. Alternatively it may arise as a result of the increase in size and frequency of the endothelial pores which occurs on the administration of the pilocarpine (7).

In the treatment of primary open angle glaucoma the drug is generally given as drops of a solution of the hydrochloride or nitrate salt at concentrations ranging from 0.5 to 4% and at a frequency of one to four times daily. Since the condition

is permanent the drops are administered for the remainder of the patient's life.

The pilocarpine eye drops suffer from various drawbacks. These include stinging of the eyes and irritation on instillation and blurring of vision. Stinging of the eyes may be due to the pilocarpine itself or to preservatives present in the formulation, for example benzalkonium chloride. Benzalkonium chloride has been reported to disrupt the superficial cells of the corneal epithelium and increase the intercellular spaces (8). It also hastens corneal drying either by disrupting the oily layer which normally spreads over the watery lachrymal film or it may encourage dissolution of the conjunctival mucin layer adsorbed on the surface of the corneal epithelium (9).

The side effects of the pilocarpine eye drops together with the frequency and duration of administration required may be a cause of the poor patient compliance observed amongst eye drop users (10, 11) and clearly indicate a case for the development of acceptable sustained release dosage forms requiring once daily administration or less for the treatment of open angle glaucoma.

1.1.3.4 Novel Ophthalmic Drug Delivery Systems

In recent years there have been various attempts at controlling the release of pilocarpine in the eye and thereby reduce the frequency of administration.

Many of the early sustained release pilocarpine formulations involved the addition of viscolysers to the eye drops. The rationale behind this was to increase the contact time of the drug with the ocular surface and thereby increase the amount of drug available for absorption by the cornea. The theory is apparent from consideration of the kinetics of pilocarpine in the eye.

On instillation of a solution of pilocarpine the amount of drug available to the eye is determined by three factors.

1. The rate of loss of the drug by drainage and by non productive absorption.
2. The permeability of the cornea to the drug.
3. The rate of disappearance of the drug from the eyeball.

Makoid and Robinson (12) suggested that rapid elimination from the precorneal area together with a rapid decrease in the concentration gradient between the tears and the corneal surface was responsible for the early peak concentration they observed in the aqueous humour, following instillation of pilocarpine nitrate drops in the rabbit eye. This rapid elimination was probably caused by the tear volume returning to its normal resident volume of 7.5µl (13) after instillation together with dilution of the drug by the normal tear flow of 0.66µl/min (14) and also by non productive absorption. It may be expected therefore that by decreasing the drainage of pilocarpine solutions by increasing solution viscosity, a marked therapeutic effect would be observed.

Chrai and Robinson (15) used methyl cellulose to increase the viscosity of a solution of pilocarpine nitrate. They showed that the viscosity of the instilled solution was related to the rate of solution drainage. They noted that over the range 1 - 15cps solution viscosity there was a linear relationship between the first order drainage rate constant and both the miotic activity and the aqueous humour drug levels attained. They reported however that there was only a relatively small increase in aqueous humour drug level for a large increase in solution viscosity. This may be explained by the hypothesis of Sieg and Robinson (16). They suggested that the cornea acts both as a barrier to pilocarpine penetration and as a reservoir for the drug. They postulated a mechanism with a small initial absorption rate in the presence of a large dose. Since the corneal epithelium is acting as a reservoir, increasing the contact time does not increase the epithelial concentration above its maximum value because the concentration gradient is unfavourable. Increasing the contact time does however maintain the epithelial concentration at a maximum value for an extended time, thus causing a shift in the peak time. This maximum value is fixed by the concentration of the applied dose.

Davies and others (17) looked at the effect of polyvinyl alcohol as a viscosity raising agent on the degree of miosis and intra ocular pressure reduction induced by pilocarpine in the human eye. Their results showed that the presence of polyvinyl alcohol to give a viscosity of 20 cs markedly increased the period of activity of the pilocarpine from about three to seven hours.

Another method which has been used to prolong the effect of pilocarpine is by the preparation of pilocarpine alginate flakes. These flakes when placed in the rabbit eye gave a significantly greater miotic response than that obtained from pilocarpine solutions (18). Restoration of normal pupillary diameter for the solid state alginate flakes was observed to occur between 7 and 8 hours after instillation as compared with 4 hours for pilocarpine alginate solution and pilocarpine hydrochloride in methyl cellulose solution.

A third method which has been developed to prolong the activity of pilocarpine in the eye is the preparation of an aqueous emulsion 'Piloplex' (19). In this emulsion the dispersed phase consists of a polymeric material to which the active ingredient is chemically bound.

An alternative approach to sustaining the release of the pilocarpine involves the use of polymeric delivery systems. Soft contact lenses, for example, prepared from 2 hydroxy ethyl methcrylate and soaked in pilocarpine hydrochloride solution have been used to deliver pilocarpine to the eye (20). At all concentrations and soaking durations tested however virtually all of the pilocarpine was released from the lenses after four hours of wear.

Possibly the most common polymeric ocular device for sustained pilocarpine delivery is the Ocusert. The Ocusert system consists of a pilocarpine core sandwiched between two transparent rate controlling ethylene vinyl acetate copolymer

membranes. It is slightly larger than a contact lens. When it is placed under the upper or lower lid of the eye the pilocarpine molecules permeate through the rate limiting membranes following zero order release over a period of several days. The Ocusert systems however have not proved successful. They are not well accepted by patients but even when in place may pin hole, releasing the entire pilocarpine content (21).

Other ocular inserts which have been developed include cellulose acetate hydrogen phthalate latices containing pilocarpine hydrochloride (22) microporous inserts and three layer inserts (23).

To date only two patents have been issued describing the use of microcapsules specifically for drug delivery to the eye. In the first (24) the microcapsules which may be of a variety of materials were embedded in a bioerodible matrix for example polylactic acid. The drug molecules released from the microcapsules diffused into the matrix and then migrated through the matrix for administration to the eye. Eventually the polylactic acid matrix would erode releasing the microcapsules. In the second patent (25), ophthalmic drugs were incorporated directly into release rate-controlled polymers which bioerode in the eye. For example chloramphenicol was encapsulated with polylactic acid and the resultant microspheres were dispersed in an aqueous ophthalmic medium. When drops of the preparation were placed in the eye the microcapsules embedded in the soft tissue lining the eye lids and gradually released chloramphenicol over 48 hours.

1.2 Microencapsulation

1.2.1 Introduction

Microcapsules are individual entities in the micron size range which are coated with a polymeric or macromolecular layer. Microencapsulation may be used to encase small particles of liquids, solids or gases with coatings which may be designed in such a way as to protect, separate, control core release or aid in storage and handling. Although most microcapsules range in diameter between 5 and 500 microns it is possible to prepare microcapsules with diameter outside these limits.

The first practical use for microencapsulation appeared in United States patents issued to Green and Schleider who used a coacervation method to encapsulate dyes in the preparation of carbonless copy paper (26) (27). In 1964 Miller and Anderson (28) were granted a United States patent for the manufacture of microcapsules containing "hydrophobic film forming polymeric wall materials dispersed in a liquid manufacturing vehicle". The first reported preparation of microcapsules by interfacial polymerisation was in 1966 by Chang and others (29). These workers prepared semipermeable polyamide microcapsules containing erythrocyte haemolysate, later publishing information concerning the use of this type of microcapsule in an extracorporeal shunt system (30).

The use of microcapsules has now spread to the food and flavour industries, for example for encapsulating fats, oils and flavours to prevent oxidation and volatilisation (31) (32). Microcapsules are used in the cosmetic industry

to encapsulate perfumes (33) and in the agrochemical industry to control the release of pesticides, e.g. methyl parathion (34). They have also been used to encapsulate activated charcoal (35), adhesives (36) and electrophotographic materials (37).

1.2.2 Techniques for the Preparation of Microcapsules

A number of techniques have been developed for the preparation of microcapsules using a variety of materials. A brief outline of the methods is given below. The work has been more extensively reviewed elsewhere. (32, 38 - 45).

1.2.2.1 Coacervation

This is the term used to describe the salting out or phase separation of lyophilic colloids into liquid droplets. Simple coacervation involves the addition of a strongly hydrophilic substance to a solution of a colloid. The added substance causes two phases to be formed. One phase is rich in colloidal droplets and the other poor in colloidal droplets. The most commonly employed simple coacervation procedures utilise gelatin as the wall forming material. In such a system coacervation is brought about by the addition of, for example, sodium sulphate or alcohol which causes deposition of the wall material as a continuous coating about the dispersed phase. The resultant microcapsules are then filtered, washed and hardened with formaldehyde before being dried. The principle requirement for simple coacervation to occur is the creation of an insufficiency of water in a part of the total system and therefore depends primarily on the degree of hydration produced.

Another process which may be used is complex coacervation. This technique is largely dependent upon the pH of the system and occurs as a result of the interaction of oppositely charged polyelectrolytes. This interaction results in the formation of a complex having such a reduced solubility that phase separation occurs. Gelatin, a protein with both carboxyl and amino groups, and acacia, a carbohydrate with carboxyl groups, are typical polyelectrolytes which can be caused to interact. A non aqueous solution of the core material is emulsified in an aqueous solution of acacia. To this is then added an aqueous solution of gelatin at a pH above its isoelectric point. On reducing the pH to 4 by the addition of acetic acid for example, the gelatin becomes positively charged and interacts with the acacia to form a complex. This complex exhibits phase separation and is deposited around the oil droplets.

Coacervation may be used for encapsulating lipophilic compounds only. Chlorthiazide (46), sulphamethoxazole (47) and thiabendazole (48) are examples of oil soluble drugs which have been encapsulated by this technique.

1.2.2.2 Non Aqueous Phase Separation

Non aqueous phase separation is one of the techniques by which water soluble substances may be encapsulated. In order to effect encapsulation by this technique an emulsion consisting of the aqueous dispersed phase containing the material to the encapsulated is prepared in an organic continuous phase containing the polymeric wall material. A second solvent, which is miscible with the first but a non solvent for the

polymer is then added. Due to the reduced solubility of the polymeric wall material in the new solvent system the wall material is forced to separate out and forms a film around the aqueous droplets. An example of a system which exhibits phase separation is an emulsion consisting of a dispersed aqueous phase and a continuous oil phase of ethyl cellulose dissolved in methyl ethyl ketone. Phase separation is brought about by the addition of polybutadiene. Aspirin is probably the most common drug which has been encapsulated by this technique (49). Another pharmaceutical which has been microencapsulated by non-aqueous phase separation methods is phenobarbitone sodium (50).

1.2.2.3 Mechanical Methods

A wide variety of mechanical methods have been developed for the production of microcapsules.

Air suspension coating: In this process the core materials, which are fluidised by an upward moving air stream, are sprayed with a coating agent dissolved in a solvent. The solution coats the fluidised particles and hot air dries the solvent.

Centrifugal extrusion: Centrifugal extrusion involves the microencapsulation of liquids using a rotating extrusion head containing concentric nozzles. In this process, a jet of core liquid is surrounded by a sheath of wall solution or melt. As the jet moves through the air it breaks into droplets of core, each coated with the wall solution. Using this process it is possible to encapsulate aqueous solutions in waxy wall materials.

Spray drying and spray congealing: Spray drying as a method of microencapsulation involves the atomisation and spray drying of an emulsion in which the core is the discontinuous phase and the wall material is a constituent of the continuous liquid phase. Spray congealing is the term used to describe spray drying when the coat is applied as a melt. Other mechanical methods developed for microencapsulation include vacuum metallizing, vapour deposition, meltable dispersion and electrostatic methods.

1.2.2.4 In Situ Polymerisation

This process involves the direct polymerisation of a single monomer on a particle surface. Particles to be encapsulated are first dispersed in a liquid vehicle such as toluene and an appropriate catalyst is added to create activated sites on the particle to be encapsulated. The monomer is then bubbled into the slurry and polymerises on the surface of the core material. An example of this process is the encapsulation of cellulose fibres in polyethylene (51).

1.2.2.5 Interfacial Polymerisation

Microencapsulation by interfacial polymerisation involves the formation of an emulsion. One monomer is present in the disperse phase and a second monomer is incorporated into the continuous phase. When the two monomers come into contact at the surface of the emulsion droplets they polymerise, forming a polymeric wall. A typical example of microencapsulation by interfacial polymerisation is the formation of polyamide microcapsules using a diacid chloride as monomer. The reaction

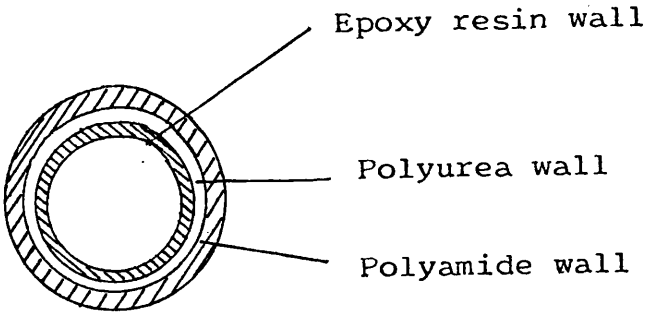
depends upon the fact that although acid halides such as sebacoyl chloride are virtually insoluble in water diamines, such as hexamethylenediamine, have an appreciable partition coefficient towards the organic phase. The polymer is therefore formed in the organic phase but is deposited almost entirely at the interface. The mechanism of the interfacial polymerisation reaction is discussed in more detail in Section 1.4. Other classes of monomers which can be used to form microcapsules by interfacial polymerisation are given in Table 1.1.

1.2.2.6 Multiple Walled Microcapsules

An extension of the microencapsulation techniques discussed is the preparation of multiple walled microcapsules. Three walled microcapsules for example have been prepared by Morris and Warburton (52, 53) using acacia and polychloroprene. An aqueous solution containing acacia was emulsified in xylene containing polychloroprene to form a water in oil emulsion which was then re-emulsified in the aqueous phase. The xylene which was trapped within the microcapsule walls could be removed after bubbling air through the multiple emulsion. This resulted in microcapsules with an aqueous core and three concentric layers of wall. The preparation of microcapsules with concentric walls of epoxy resin, polyurea and polyamide has also been reported (54). In this process an aqueous solution of polyamine was dispersed in toluene. Solutions of epoxy precondensates polyisocyanate and polyacid chloride were then successively added to the toluene phase to cause interfacial polymerisation in a three layer structure. (Figure 1.2)

Figure 1.2

Multiple Walled Microcapsule



1.2.2.7 Nanocapsules

A further extension of microencapsulation is the preparation of nanocapsules, microcapsules in the nonometer size range. The preparation of the nanocapsules is undertaken with micellar systems. An aqueous solution of the material to be encapsulated is solubilised in a weakly polar vehicle to yield micelles of water in an organic outer phase. An amphiphilic monomer is then added to the lipophilic external phase and is enriched at the interface to form a mixed micelle. The monomer is then polymerised with the aid of suitable procedures for example gamma radiation, heat and chemical agents to produce solidified hardened micelles with drug molecules entrapped. Finally, the outer lipophilic vehicle is replaced by water.

REACTING GROUPS			LINKING STRUCTURE	POLYMER
-O-H Bisphenol	+	Cl-C- Dicarbonyl Chloride	-O-C-	POLYESTER
-O-H Bisphenol	+	O=C=N Di-isocyanate	-O-C-N-	POLYURETHANE
-O-H Bisphenol	+	Cl-S-O Disulphonyl Chloride	-O-S-	POLYSULPHONATE
-O-H Bisphenol	+	Cl-C-Cl Phosgene	O-C-O	POLYCARBONATE
-N-H Diamine	+	Cl-C- Dicarbonyl Chloride	-N-C-	POLYAMIDE
-N-H Diamine	+	Cl-S-O Disulphonyl Chloride	-N-S-	POLYSULPHONAMIDE

TABLE 1.1 CHEMICAL CLASSES OF POLYMERS THAT MAY BE PREPARED BY INTERFACIAL POLYCONDENSATION.

1.3 Release Characteristics of Microcapsules - Theoretical Considerations

The term microcapsule is generally used to describe a hollow sphere of polymeric material with encapsulated drug contained in solution or suspension within the core. This type of system is termed a reservoir device. In the majority of capsule type delivery systems the polymers are non-porous and drug traverses the membrane by a process involving solute dissolution in the membrane structure followed by solute diffusion along and between polymer segments and is finally released into the external medium. Thus the release of drug from a capsule type device is a permeation process and in the case in which the drug is dissolved in the core the situation is analogous to the diffusion of drug through a planar membrane (55). From a consideration of Ficks law of diffusion which defines the flux across a plane surface and may be written as equation 1.1, it can be seen that as drug is released from the microcapsules so the concentration gradient decreases and therefore the rate of drug release decreases.

$$\frac{dM}{dt} = \frac{PA\ (C_s - C_b)}{x} \qquad \text{(equation 1.1)}$$

where $\frac{dM}{dt}$ is the rate of drug release

P is the permeability constant for a given drug and membrane

A is the surface area of the microcapsule

x is the membrane thickness

C_s and C_b are the concentrations of drug in the encapsulated and bulk (sink) solutions respectively

Equations governing release under changing concentration gradient for microcapsules in which the thickness of the microcapsule wall is much smaller than the core radius have been proposed by Benita and Donbrow (56). Under sink conditions in which the sink solution

volume is much larger than the internal volume of the microcapsules the equations derived simplify to give equation 1.2.

$$M_t = M_o \exp - \frac{PAt}{xV_1} \qquad \text{(equation 1.2)}$$

where M_t and M_o are the mass of drug in the microcapsules at time t and time 0 respectively

V_1 is the internal volume of the microcapsule

In the case in which the drug is dispersed in the microcapsule core, that is the drug loading is greater than the drug solubility the microcapsule behaves as a true reservoir device. The drug traverses the microcapsule membrane by a diffusion process following dissolution in the core vehicle. The undissolved drug therefore acts as a constant source of solute available for diffusion. The release is again governed by Ficks law which has been modified by Baker and Lonsdale (57) to relate to the release from a spherical reservoir of radius r_1, surrounded by a spherical membrane of radius r_o. (equation 1.3).

$$\frac{dM}{dt} = 4\pi \, DK \, \Delta C \, \frac{r_o r_1}{r_o - r_1} \qquad \text{(equation 1.3)}$$

where D is the drug diffusion coefficient in the membrane

K is the drug partition coefficient in the membrane

ΔC is the concentration gradient across the membrane

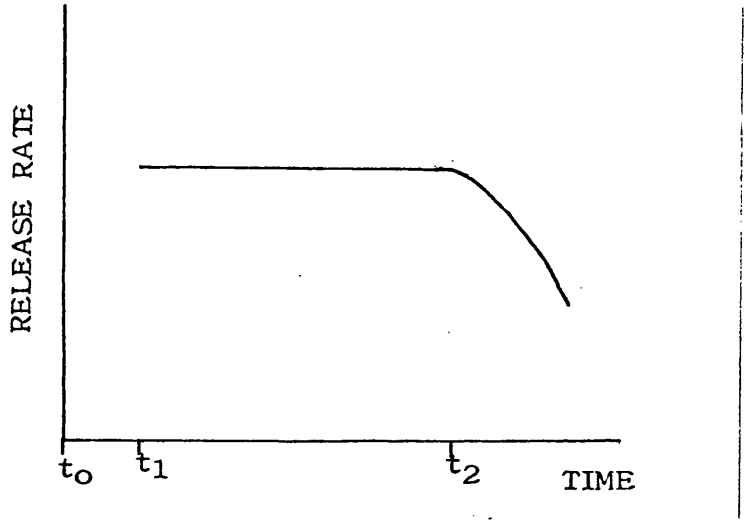
Mathematical expressions defining the cumulative amount of drug released from unit surface area of a capsular reservoir system were derived by Chien and Lambert (58). It was proposed that if sink conditions were maintained throughout and there were no diffusion effects in the boundary layer, that is release was governed by diffusion through the polymer, then the rate of drug release was defined by equation 1.4.

$$\frac{dM}{dt} = \frac{C_s D}{x} \qquad \text{(equation 1.4)}$$

where C_s is the solubility of the drug in the polymer phase. From equation 1.4 it is seen that the release rate is independent of time but is a linear function of both the solubility of the drug in the polymer and the membrane diffusivity. The release from such a device will therefore exhibit zero order kinetics over a time period t_1 to t_2 which is illustrated diagrammatically in Figure 1.3.

Figure 1.3

Typical Plot of Release Rate versus Time for a Reservoir Device



During the initial period t_o to t_1 the release rate will not be constant. For example, if the system is loaded with drug and used immediately some time will be required before a concentration gradient is established within the membrane and a lag time effect will be observed. Conversely if a device is stored for some amount of time before release, the membrane will be saturated with drug and the initial release rate will be high. At some time t_2 the mass of drug per unit volume in the core will approach the solubility of the drug. At this point the release characteristics will be

those exhibited by a solution containing device according to equation 1.2.

The term microcapsule is also used to describe a polymer matrix in the micron size range containing drug dissolved or dispersed as solid throughout. This type of delivery system is known as a matrix device. In the case of a delivery system containing drug dissolved in the polymer matrix the drug traverses the polymer by diffusion and is desorbed. During the release process drug is lost from the outer layer of the microcapsule. Therefore as time continues the diffusion path increases since the outer layers become depleted of drug and the remaining solute must diffuse through these outer layers before being released. From a consideration of Ficks equation written as equation 1.5 it may be seen that the concentration gradient and therefore the flux is time dependent for a matrix type device.

$$J_d = -D \frac{dc}{dx} \qquad \text{(equation 1.5)}$$

where Jd is the flux across a plane surface of unit area

$\frac{dc}{dx}$ is the concentration gradient of the solute across the diffusion path dx

The effects of various parameters on the release rate of matrix devices of different geometrics have been considered by Baker and Lonsdale (57). For a sphere the rate of release during early and late time periods was given by equations 1.6 and 1.7 respectively.

$$\frac{\frac{dM_t}{M_\infty}}{dt} = 3\left(\frac{D}{r^2 \pi t}\right)^{\frac{1}{2}} - \frac{3D}{r^2} \qquad \text{(equation 1.6)}$$

$$\frac{dM_t/M_\infty}{dt} = \frac{6\ Dt}{r^2} \exp\left(\frac{-\pi^2\ Dt}{r^2}\right) \qquad \text{(equation 1.7)}$$

where M_∞ is the total amount of drug in the polymer

M_t is the amount of drug desorbed at time t

r is the radius of the device

In the case in which the drug is dispersed as a solid in the polymer matrix the kinetics are altered. The mathematical model describing dispersed drug release from a polymer by a solution-diffusion mechanism has been described by Higuchi (59, 60) and the amount of drug released is given by equation 1.8.

$$M_t = A \left\{ DC_s\ (2\ C_a - C_s)t \right\}^{\frac{1}{2}} \qquad \text{(equation 1.8)}$$

where C_s is the solubility of the drug per unit volume in the matrix

C_a is the total amount of drug present per unit volume

In this case the amount of drug released is linear with time to the power one half. Therefore the rate of release decreases with time. In the situation where the drug is dispersed in the polymer but diffuses through channels or pores in the polymer rather than by a solution diffusion mechanism it is necessary to modify equation 1.8 to take account of the tortuosity and porosity of the matrix.

In the case of microcapsules with microporous membranes or those in which a partially soluble membrane encloses a drug core the kinetics of release are again different. In the case of microporous membranes the rate of a solutes permeation through the membrane will depend on the pore size and the solutes molecular volume. In the second type of capsule the dominant factor will be the fraction of soluble polymer in the coat. An alternative type of

controlled release capsule is one controlled by dissolution. An example of this is microencapsulated drug in which all the drug is in the core and complete erosion of the coat leads to an abrupt release of contained drug. In this instance the time taken for release of the drug is governed by the dissolution rate constant for the polymer and the coating thickness. The release kinetics from an erodible solid sphere with solute dispersed throughout have been examined by Hopfenberg (61), who has shown that since the radius of the sphere is ever decreasing a continuously decreasing release rate results.

1.4 Polyamides

1.4.1 The Synthesis and Structure of Polyamides

Nylon is the generic name given to a series of polymers known as polyamides. The first nylon to be developed commercially was the condensation product of hexamethylenediamine and adipic acid. It was called Type 6.6 nylon, the name being derived from the number of carbon atoms in the parent diamine and diacid monomers. Later a different method of making polyamides was discovered, namely by condensation of certain W-amino acids. Nylons prepared by this method are described by a single number which is derived from the number of carbon atoms in the parent amino acids. Examples of the synthesis of polyamides are given below.

1. Condensation of a Diacid and a Diamine

$$n\ HOOCXCOOH + n\ H_2NYNH_2 \rightarrow n \begin{bmatrix} COO^- \\ | \\ X \\ | \\ COOH \end{bmatrix} + \begin{bmatrix} {}^+NH_3 \\ | \\ Y \\ | \\ NH_2 \end{bmatrix}$$

$$\rightarrow \left\{ OCXCONHYNH \right\}_n + 2(n-1)H_2O$$

Nylon 6.6 and nylon 6.10 are synthesised by this method using hexamethylenediamine and adipic acid or sebacic acid respectively. There are a large number of other diamines and diacids which can be used to prepare polyamides by this route ranging from ethylenediamine and phthalic acid to BIS (3-aminophenyl)methyl phosphine oxide and

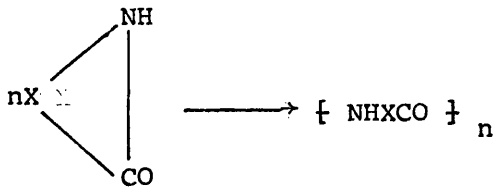
BIS (4 carboxyphenyl)-methyl phosphoric acid (62).

2. Self-condensation of an ω-Amino Acid

$$n\ NH_2XCOOH \rightarrow \{\ NHXCO\ \}_n + n-1\ H_2O$$

This is the method used to prepare nylon 11 from W-amino-undecanoic acid. A suitable catalyst is phosphoric acid.

3. Scission of a Lactam Ring

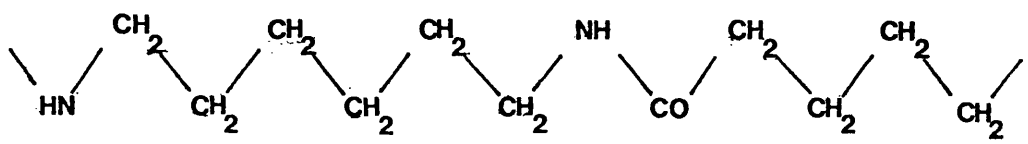


Nylon 6 is prepared from ε-caprolactam by this route using water as a catalyst. Nylon 12 is also prepared by this method from laurilactam.

The general structure of the polyamides is that of alternating hydrocarbon and imido groups as typified by nylon 6.6 (Figure 1.4).

Figure 1.4

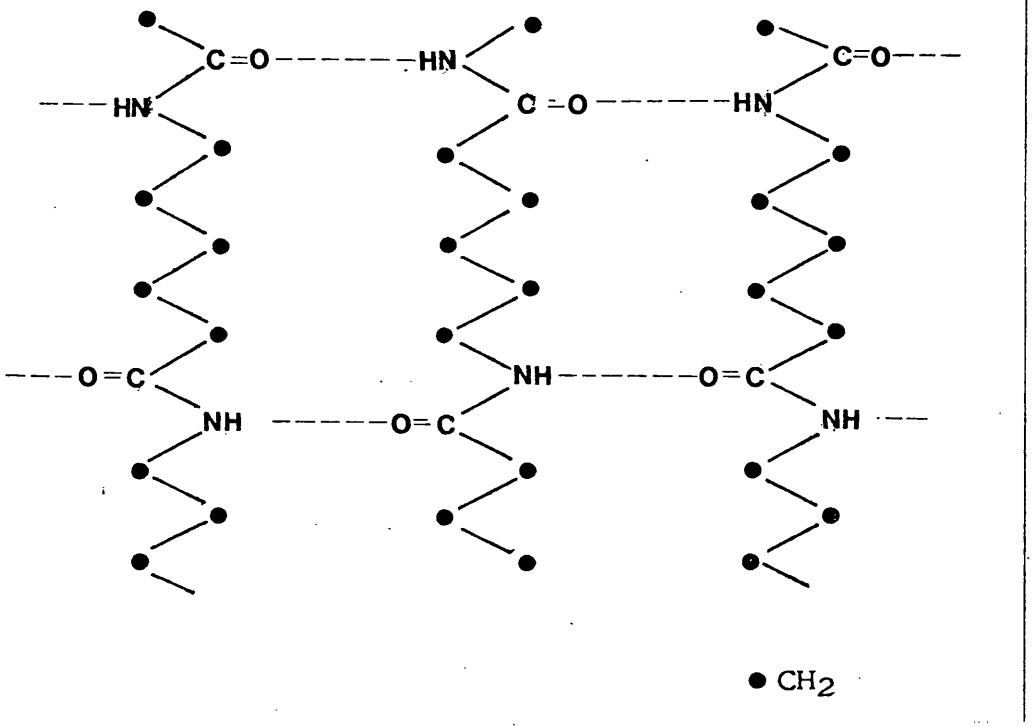
Schematic Representation of Nylon 6.6



The polymer matrix is heterogeneous in nature consisting of both amorphous and crystalline regions. Within the crystalline regions the presence of the polar imido groups leads to a strong attraction between the molecules due to hydrogen bonding. The way in which these interchain hydrogen bonds may form is shown schematically in Figure 1.5.

Figure 1.5

Schematic Representation of Hydrogen Bond Formation between Nylon 6 Molecules



Although the alignment of chains as represented above is only strictly found in the crystalline regions where intermolecular hydrogen bond formation will be at a maximum, hydrogen bonding does occur in the amorphous regions but to a more limited extent. The proximity of the amide chains and thus the extent of bond formation is influenced by environmental factors such as physical stress (63) hydration (64) and

temperature (65). Chemically, within the polymer matrix, there are polar and non polar regions which correspond to the local relative abundance of amide groups and methylene chains.

1.4.2. Properties of Polyamides

The high degree of intermolecular attraction in the crystalline regions of polyamides results in high melting points and high solubility parameters. Only solvents capable of specific hydrogen bond formation with the polymer effect solution of polyamides at room temperature, for example, formic acid.

The properties of polyamides are readily altered by varying the hydrocarbon chain length or by substitution of groups. For example, the longer the hydrocarbon chain the lower will be the attractive forces between the polymer chains, as a result of the reduced number of hydrogen bonding amide groups. This results in a lowering of the melting point. Substituting an alkyl group for a hydrogen in the hydrocarbon chain also lowers the melting point and increases the solubility of the polymer. This occurs as a result of the presence of the alkyl group which prevents the close approach of the molecules thus reducing hydrogen bonding between adjacent chains.

Hydration of the polyamide matrix occurs by specific bond formation between water molecules and unassociated amide groups. Water penetrates the polymer matrix via the amorphous regions but cannot gain access to the relatively denser crystalline

regions. Three types of water-amide group bond have been postulated (66) giving rise to three possible 'types' of water being present in polyamides:

1. Tightly bound water in which the molecules from a double hydrogen bond between the carboxyl free electron pairs of two adjacent amide groups.

2. Loosely bound water where the molecules can bind by joining existing hydrogen bonds between the carboxyl and amide groups of adjacent amide links.

3. Capillary condensed water which occurs by the self association of incoming water molecules with existing bound water.

1.4.3 Interaction of Solutes with Polyamides

1.4.3.1 Classification of the terms used in Solute-Sorbent Interactions

The various types of solute-sorbent interactions which may occur when an adsorbent is placed in a solution of a solute are listed below.

a) Adsorption: This term refers to the transfer of solute from bulk solution to the interface between the two phases of the sorption system.

b) Sorption: is the term applied to the interaction whereby the solute penetrates into the bulk of the sorbent by crossing the interface between the sorbent and the bulk phase.

c) Persorption describes the process whereby solute molecules penetrate a series of pores in the bulk of the sorbent, as opposed to sorption which occurs at a molecular level within the sorbent.

d) Permeation is the term used to describe the process in which solute molecules cross a sorbent which separates a solute solution from a second bulk phase. The mechanism of permeation takes place by sorption, distribution and diffusion processes.

e) Desorption is the term applied to the process whereby the net transfer of solute occurs from the sorbent back into solution. It applies to the reverse of adsorption, sorption and persorption.

1.4.3.2 Polyamide - Solute Interactions

The interaction between polyamides and weak organic acids and bases in the unionised form is generally considered at the molecular level to be a sorption process. These interactions are typified by linear (C Type) adsorption isotherms (67, 68) which are representative of systems where the solute is capable of penetrating the sorbent. They may therefore be considered as akin to a partition process.

The nature of the interaction between solutes and polyamides has been the subject of several reports in the literature. It has been suggested that these interactions may involve ionic attractions, Van der Waals' forces or hydrogen bond formation. Ionic forces, for example, are usually involved when the ionised form of organic acids or bases are sorbed by polyamides (69). The uptake of organic acids such as formic acid however involves only forces of the Van der Waals' type (70), whereas the interaction between weak organic acids such as sorbic acid and polyamides have been reported to involve

hydrogen bond formation (71). Kapadia and others (72) investigated the interaction of a number of 4-hydroxybenzoate derivatives with polyamides. They proposed that these solutes formed very weak hydrogen bonds with the amide groups in the polymer which were then stabilised by Van der Waals' forces. Later Richardson proposed that the sorption of ethyl-4-aminobenzoate by nylons may also be related to the swelling of the matrix by water (67).

Consideration of the structure of the nylon matrix, namely the presence of amorphous and crystalline regions, hydration of the amorphous areas and the presence of polar and non polar regions, led Wicks (73) to propose three possible mechanisms of binding of solutes in polyamides.

1. A solute, unable to form hydrogen bonds directly with the polymer, may associate with the hydrophobic regions present by the formation of weak Van der Waals' forces.
2. Solute molecules which are capable of forming weak hydrogen bonds with the polymer, but are unable to displace bound water from the amide linkages, may form an association with this bound water. In this case sorption of the solute would depend upon the degree of hydration of the matrix.
3. Direct association of a solute with the polyamide can arise in two ways. A solute capable of forming moderately strong hydrogen bonds could displace loosely and tightly bound water from the amide groups in the amorphous regions of the matrix and hydrogen bond directly. If the solute was not capable of forming sufficiently

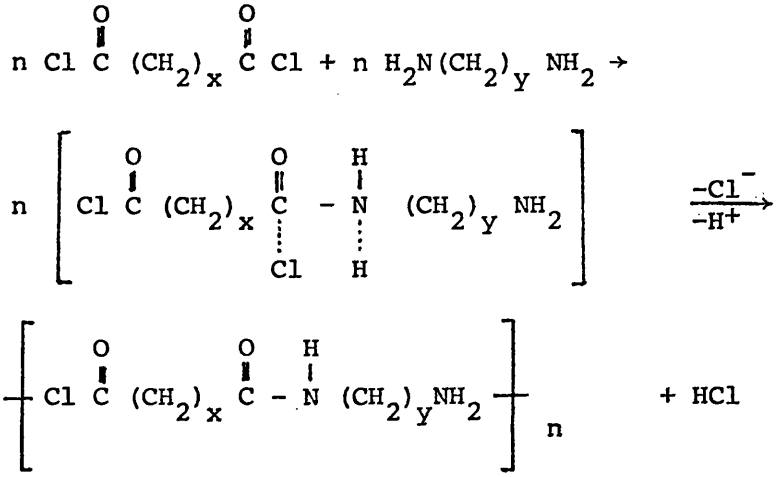
strong hydrogen bonds to break the interchain hydrogen bonds in the polymer access to the highly crystalline regions would not occur. If, however, the solute was able to break the interchain hydrogen bonds and gain access to the crystalline regions the result would be eventual plasticisation and dissolution of the polymer.

1.5 Polyamide Microcapsules

1.5.1 The Interfacial Polymerisation Reaction in the Formation of Polyamides

1.5.1.1 Reaction Mechanisms

Interfacial polymerisation may be defined as a polymerisation reaction which takes place at the interface of two or more immiscible liquids. For example, at the interface between a water layer containing a diamine and an oil layer containing a diacid chloride, polycondensation will occur to form a film of polyamide with the release of hydrogen chloride.



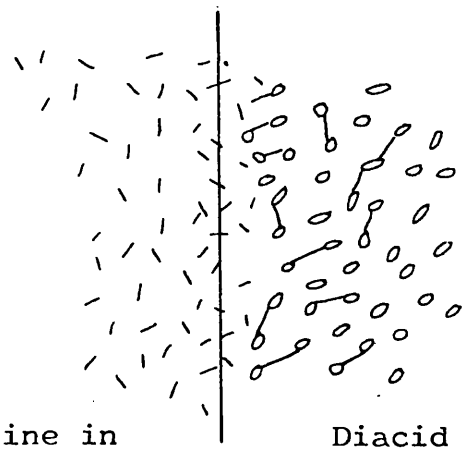
The reaction is believed to take place by an S_N2 mechanism to form a protonated amide from which a proton is rapidly eliminated by the presence of an added base (74, 75). It has been shown that reactions between acid chlorides and nucleophiles proceed essentially irreversibly (76). Reaction rates of polycondensation reactions vary widely from 10^1 to 10^5 litre $mole^{-1}$ sec^{-1} (77). In the case of piperazine reacting with phthaloyl or terephthaloyl chloride the rate constant has been reported to be 10^4 to 10^5 litre $mole^{-1}$ sec^{-1} (78).

When a solution of the diacid chloride is brought into contact with the aqueous phase containing the diamine high molecular weight polymer forms rapidly. It is generally accepted that the polymer forms on the organic side of the interface while the acid by product accumulates as a salt in the aqueous phase.

Upon phase contact both reactants and solvents tend to become partitioned to some extent into the opposite phase and the success of the reaction depends upon this (Figure 1.6). The diamine nearly always has an appreciable potential partition toward the organic phase whereas the acid chloride has very little solubility in water. Partition equilibria in diffusion controlled systems however are never achieved during polymerisation as acylation takes place in the organic phase as rapidly as diamine is transferred. Were this not so, the diamine would have time to penetrate more deeply into the acid chloride layer and reaction and polymer precipitation would take place more diffusely, as occurs in kinetically controlled systems (77).

During interfacial polycondensation the first diamine molecules meet a high concentration of acid chloride and are acylated to a large extent at both ends. The following diamine molecules find a layer of acid chloride terminated oligomers (that is polymers with few monomer units) plus diacid chloride and the reaction proceeds by an irreversible coupling of the oligomers by the diamine.

a.



Diamine in water

Diacid chloride in organic solvent

Interface

b.

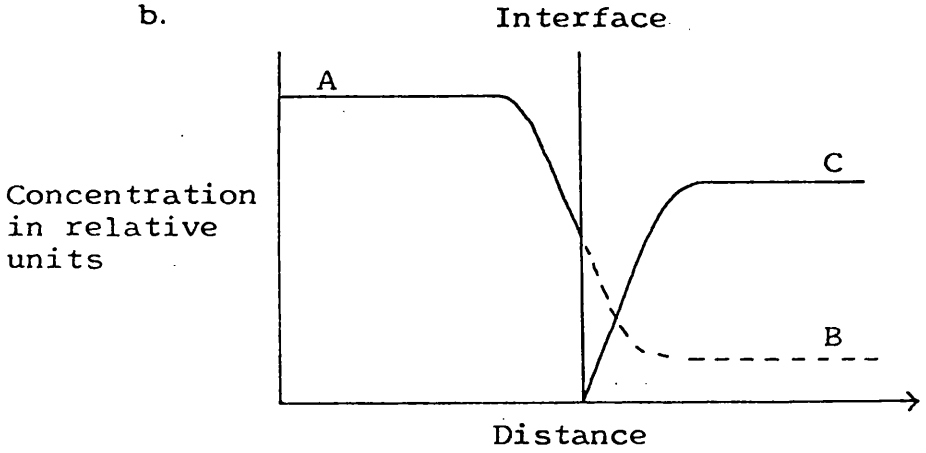


FIGURE 1.6 DIAGRAM OF RELATIONSHIP OF REACTANTS IN INTERFACIAL POLYMERISATION.

a. — Diamine molecules
o Diacid chloride molecules

b. A Amine groups in water phase
B Amine groups at partition 'equilibrium' in organic phase
C Acid chloride groups in organic phase

The concentration and size of the oligomers increases until a layer of high polymer is obtained. High polymer forms because of the increasing probability that diamine reacts with an oligomer rather than new diacid chloride.

The reaction therefore may be considered to take place in a series of incremental layers through which the diamine advances from the liquid interface and into the organic phase. As the concentration of polymer species increases interchain contacts increase until an intact network is formed. Polymerisation however does not entirely stop at this stage but is greatly decreased in rate. This is because of the lower mobility of the polymer chains and the decreased diffusion rate of the intermediates. It may also be due to hydrolysis of the acid chloride (79). It has been observed experimentally that as the diamine diffuses it carries with it some water into the organic phase. This water hydrolyses the reactive acid chloride which cannot now react with the diamine and therefore may result in inhibition of further growth of the membrane.

$$R - COCl + H_2O \rightarrow RCO_2H + HCl$$

During the course of the reaction the ratios of the reactants within the layers of the polymer vary. Therefore there may exist a gradation of average molecular weights across the width of the film which would vary with the original concentration of the reactants. It has also been suggested that as the membrane thickness increases so the structure of the membrane becomes more and more disordered (79). Thus the

membrane will have two surfaces; a smooth surface near the water phase and a rough surface near the organic phase. The same workers also reported that the polymer chains are mainly oriented perpendicular to the interface (79) and because of the growth mechanism both surfaces will expose different end groups. They also suggested that there was a continuous change in the concentration of type of end group across the membrane.

To obtain the highest polymer, the balance of transfer rates of reactants must be such that equivalents of reactants enter the polymerisation zone just within the solution period. For this reason, the system is sensitive to those factors which change the transfer rate of diamine to the organic phase as well as to changes in the reaction rate and solvent swelling of the polymer.

1.5.1.2 Factors Affecting Polymer Yield

The factors affecting the yield of polymers formed by interfacial polycondensation have been proposed by Morgan and Kwolek (80, 81) and MacRitchie (82) for nylon 6.10 and by Bradbury (83) for polyterephthalamide.

1. Chemical Reaction Rate: The chemical reactions employed in interfacial polycondensation are often extremely fast having rate constants of the order of 10^2 to 10^4 litre $mole^{-1}$ sec^{-1}. The reaction rates are dependent upon the nature of the reactants used (77). The basic constraint on the rate however is that it must be faster than any important side chain reactions which occur at

the polymerisation site such as hydrolysis of the acid chloride (79) and incorporation of monofunctional monomers. The polymerisation must also be complete before the polymer is immobilised by precipitation.

2. Precipitation Rate of the Polymer: The precipitation rate of the polymer is important in terms of the chemical reaction rate in that precipitation must not occur before the reaction is complete. It is also important that the precipitation rate is such that the polymer should stay in solution for a sufficient period to attain a high degree of polymerisation.

3. Material Purity: A high grade of purity of reactants and solvents is a requirement for the preparation of polyamides of high molecular weight by interfacial synthesis. The presence of monofunctional compounds will result in the termination of propagating molecules (84) whereas polyfunctional compounds will give rise to branching and crosslinking. Mono or polyfunctional compounds may be formed on storage of reactants as a result of hydrolysis, oxidation and so on. Some impurities may be tolerated in interfacial polycondensation provided they react more slowly than the polymer intermediates. They may also be tolerated if they are withheld selectively from the polymerisation zone by the aqueous phase. Slow reacting impurities however, may accumulate in the polymerisation phase and compete successfully with polymer intermediates in the formation of final polymer. This would result in a lowering of the molecular weight.

4. Hydrolysis of the Acid Chlorides: Acid chloride hydrolysis occurs primarily in the aqueous phase and since most diacid chlorides have low solubility in water they are protected to a large extent. Of the diacid chlorides, the shorter chain aliphatic molecules are the most water soluble and are therefore hydrolysed to the greatest extent (81) resulting in a depletion of the reactant. It has also been reported that some water is present in the organic phase (79) and therefore some hydrolysis must occur at the interface and in the bulk of the organic phase. Any hydrolysis products which are formed will be water soluble and therefore will be held out of the polymerisation region by the aqueous phase.

5. Stirring Rate: Stirring rate is important in relation to the generation of 'interfacial area' (85). The interfacial surface area provides the points of contact for the reacting molecules. Additionally, the rate of mass transfer of material across films adjacent to the interface is directly proportional to the interfacial area as well as to the concentration gradient. The effect of stirring rate on the molecular weight of the polymer produced is variable. It has been suggested that the effect of stirring is to increase the availability of the diamine (81). Therefore, in a system in which the diamine concentration in the organic phase is below the optimum concentration for the relative amount of diacid chloride present, increasing the stirring speed will increase the molecular weight of the polymer formed. Conversely for a system in which

the concentrations of the monomers in the organic phase are at an optimum for polymer formation, increasing the stirring speed will decrease the molecular weight of the polymer formed. This will occur due to the creation of a relative deficiency of diacid chloride molecules in the organic phase arising as a result of the increased availability of the diamine.

6. Effect of additives and pH: Small amounts of detergents are sometimes helpful to stirred polycondensation reactions. This is due to improved mechanical mixing and contact of the reactants which may result in the formation of smaller droplets of dispersed phase. Surface active agents may also assist the transfer of both diamine and salt across the interface.

The presence of salts such as acid acceptors strongly affects the polymerisation. Neutral salts, in particular, shift the partition of the diamine toward the organic phase. This is probably due to a change in the amount of unionised diamine, as well as salting out effects. Changes in the pH of the aqueous phase will also affect the relative amounts of ionised and unionised diamine. Salts in the aqueous phase also reduce the solubility of the acid chloride in the water thereby reducing any hydrolysis. The addition of sodium bicarbonate as an acid acceptor (29) is detrimental to the polyermisation as it is a weak base. When acted upon by an acid it produces carbon dioxide which in turn withholds diamine from the reaction resulting in

lowered yield and molecular weight. Bradbury and others (83) looked at the salt effect on the rate of reaction between terephthaloyl chloride with piperazine. They considered that the observed changes in rate constant with varying salts and salt concentrations was due to the salting in or salting out of amine from the aqueous phase and acid chloride from the organic phase.

7. Phase Volume and Monomer Concentration Ratios: The viscosity of the polymer obtained varies according to the relative amounts of each monomer present in the polymerisation zone at a given time. This is dependent upon the phase volumes and monomer concentrations used. On increasing the relative amount of diamine present the diffusion of the diamine into the organic phase will increase with the result of the formation of a less compact polymer. Increasing the relative amount of diacid chloride however, will increase the concentration of this monomer in the polymerisation zone, restricting the thickness of the film formed.

8. Choice of Organic Solvent: The choice of organic solvent determines the partition coefficient of the diamine. As the consequence of a change in the organic solvent therefore, there will be a shift in the reactant concentration ratios in the polymerisation zone. This will result in a change in the molecular weight of the polymers obtained. Additionally the better the solvent for the polymer the higher the molecular weight of the product

due to the greater time for which the polymer will be in solution before precipitation occurs.

9. Method of reactant addition: It has been suggested that the mixing of the organic and aqueous phases should be as rapid as possible (81). If the acid chloride, for example, is added slowly the first portion will react with an excess of diamine resulting in a lowering of the molecular weight of the polymer.

1.5.1.3 The Interfacial Polymerisation Reaction in Microencapsulation

The factors which influence the interfacial polymerisation reaction discussed in Section 1.5.1.2 will also affect the formation of microcapsules using this method. For example, it has been found that because of the increased water solubility of the shorter chain aliphatic diacids and therefore their increased hydrolysis, microcapsules cannot satisfactorily be prepared from diacid chlorides where the chain consists of less than eight carbon atoms (86). It is also probable that the properties of the microcapsules and the microcapsule membranes will be affected by all those factors influencing the partition of the diamine into the organic phase. This has been found to be true, for example, in the case of the presence and nature of any emulsifier (87, 88), solvent composition (87), pH of the aqueous phase (88) and the nature of the diamine (87, 89). The effect of each of these conditions on the microcapsules formed is discussed in Section 1.5.3.

In particular, the choice of any core material to be encapsulated is important as it may interfere with the polymerisation reaction. This may occur not only by affecting the partition of the diamine but also by reacting with either of the monomers. Aniline, for example, has a high affinity for the organic phase and when included as a core material hinders the interfacial reaction by reacting with the acid chloride and terminating propogation of the chains (54). Some quarternary ammonium compounds have also been found to interfere with the polymerisation reaction preventing the formation of microcapsules (90).

1.5.2 The Preparation of Polyamide Microcapsules by Interfacial Polycondensation

1.5.2.1 Original Method

The preparation of nylon 6.10 microcapsules containing erythrocyte haemolysate was first described by Chang and others in 1966 (29). Details of this method are given below and are shown schematically in Figure 1.7.

Stage I. To 1.5ml of haemolysate in a 100ml beaker was added an equal volume of 0.4M hexamethylenediamine in 0.45M carbonate-bicarbonate buffer at pH9.8. The buffer was present as an acid acceptor to neutralise the hydrogen chloride formed during the polymerisation reaction. The beaker was surrounded by ice. This may have been to counteract the heat formed during the polymerisation possibly denaturing the protein. It may also have had the effect of slowing any hydrolysis of the diacid chloride (91). The buffered haemolysate was then immediately emulsified for 1 minute with 15ml of a "mixed solvent" (chloroform : cyclohexane 1:4 containing 1% v/v Span 85).

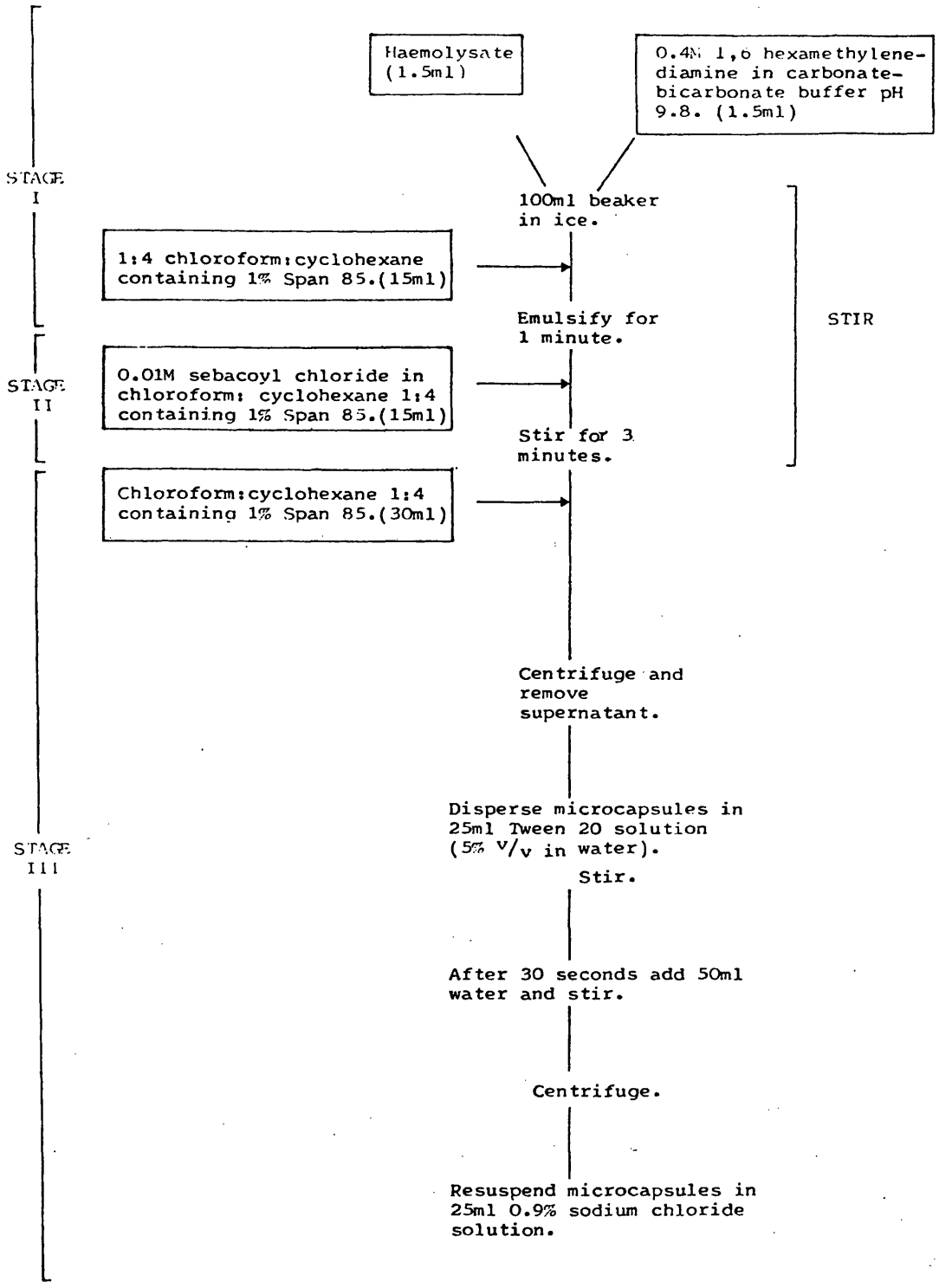


FIGURE 1.7 SCHEMATIC REPRESENTATION OF NYLON 6.10 MICROCAPSULE PREPARATION ACCORDING TO THE METHOD OF CHANG AND OTHERS (29).

Chang and co-workers noted that although many other solvent phases could be used, that particular combination satisfied the following requirements.

(1) The liquid should be one which does not readily denature proteins, as protein was the core material, e.g. cyclohexane, carbon tetrachloride and chloroform.

(2) It is desirable to have a liquid with specific gravity fairly close to unity as with denser liquids the formed microcapsules would tend to rise to the surface and consequently be less easily separated by centrifugation. With liquids of lower specific gravity the microcapsules would sediment rapidly and become harder to redisperse.

(3) The partition coefficient of the organic liquid for the diamine determines the properties of the membrane (80). Chloroform which has a high affinity for diamine produces strong but coarse and thick membranes whereas cyclohexane produces smooth and very thin but weak ones. The two solvents combined in the stated proportion resulted in an organic phase with a satisfactory specific gravity which also allowed the production of thin but satisfactorily strong membranes.

No indication was given as to the selection of surface active agent. Span 85 was presumably used as it produced a stable water in oil emulsion using the above disperse and continuous phases. Emulsification was effected using a magnetic stirrer and 4cm stirring bar. The concentration of surfactant and stirring speed were possibly selected so as to produce microcapsules of the desired size.

Stage II. Without stopping the stirring, 15ml of sebacoyl chloride solution was added and stirring continued for 3 minutes. The sebacoyl chloride solution was prepared immediately before use by adding 0.1ml of pure sebacoyl chloride to 25ml of the mixed solvent to give a concentration of 0.018M. It was necessary to prepare this solution immediately before use in order to decrease the possibility of hydrolysis of the acid chloride. The stirring time was also important. If the reaction time is too brief a leaky macroporous membrane is formed whereas prolonged contact of diamine with diacid chloride yields a thick membrane with an excess of low molecular weight polymer on the organic liquid side. Removal of the microcapsules from the reaction mixture was therefore carried out rapidly.

Stage III. At the end of the reaction time 30ml of the 'mixed solvent' was added to the stirred suspension. The addition of the solvent may have had the effect of quenching the reaction by diluting the system. The suspension was then centrifuged at 350g for 15 seconds and the supernatant discarded; possibly at high spin speeds the microcapsules are likely to rupture. The microcapsules were then dispersed in 25ml of 50% v/v aqueous Tween 20 solution. Tween 20 was used as it is capable of forming a stable oil in water emulsion and it assists in separation of the organic phase associated with the microcapsules. Chang and others (29) noted that the concentration of the surfactant was important as at lower concentrations the microcapsules were not completely separated from the organic phase. Dispersal of the microcapsules was

achieved first by gentle stirring with a glass rod and then using a magnetic stirrer. 50ml of water was added and the suspension stirred for 30 seconds, centrifuged and the supernatant discarded. The microcapsules were then quickly resuspended in 25ml 0.9% sodium chloride solution. Chang and others (29) noted that the microcapsules at this point may have been crenated due to their exposure to hypertonic Tween 20 solution but that they regained their sphericity in the saline if the period of contact with the strong Tween 20 medium had been kept as short as possible.

Photographs of microcapsules prepared by this method show that they had the appearance of hollow spheres with clear interiors (29). Their size ranged between 20μm and 80μm diameter depending on the Span 85 concentration and the stirring speed used. It was estimated that the membrane thickness of the 80μm diameter microcapsules was approximately 0.2μm. It was also noted that the microcapsules were flexible and under hydrostatic pressure a 100μm diameter microcapsule would pass through a capillary half that diameter.

1.5.2.2 Modifications to the Original Method

The above method developed by Chang and his colleagues (29) has been modified by a number of workers to produce microcapsules containing different core materials or with different physical properties. Parameters which have been modified include the following:

1. Solvent System. The majority of microcapsule preparations in the literature utilise chloroform and cyclohexane in

various ratios as the solvent system. Shiba and others (92) Koishi and others (37) and Jenkins and Florence (93) all used an organic phase consisting of cyclohexane and chloroform in the ratio of 1 to 3. Aisina and co-workers (94) prepared polyamide microcapsules using an organic phase also consisting of cyclohexane and chloroform but in the ratio of 1 to 5. The polyterephthaloyl microcapsules prepared by Ishizaka and others (95) were formulated using chloroform to cyclohexane ratios ranging from 1 to 2, to 1 to 6. Degennaro and colleagues (96) however in the preparation of nylon 6.10 microcapsules used carbon tetrachloride as the organic phase.

2. Surface Active Agent. Span 85 is the most often used surfactant in polyamide microcapsule preparation. The original concentration used by Chang and co-workers (29) to prepare microcapsules of 80μm diameter was 1% v/v. It was noted however, that in order to prepare smaller microcapsules of approximately 5μm diameter using an homogeniser it was necessary to increase the concentration of Span 85 to 15% v/v to prevent aggregation of the microcapsules. Other workers have used Span 85 concentrations ranging from 2% v/v to 20% v/v (87, 89, 97, 98). Although in the majority of reports no reason is given for the selection of surfactant concentration, it may have been to prepare microcapsules of a given size. Luzzi and others (99) used Brij 52 at a concentration of 1% v/v as the surfactant in the preparation of polyamide microcapsules and Jenkins and Florence (93) used 10% Arlacel A. Again no reason for

the choice of surfactant was given but presumably both surfactants resulted in the formation of a stable water in oil emulsion.

3. Monomers. A number of diamines and diacid chlorides have been used in the preparation of polyamide microcapsules. Takamura and others (100) for example, prepared microcapsules from various combinations of the following diamines and diacid chlorides: phenylenediamine, piperazine, 2,5 dimethyl piperazine, 1,6 hexamethylenediamine with sebacoyl chloride or phthaloyl dichloride. Koishi and colleagues (87) used phthaloyl chloride in combination with either 1,6 hexamethylenediamine or piperazine. Ishizaka and co-workers (95) prepared microcapsules using piperazine and terephthaloyl chloride.

4. Phase Volume and Monomer Concentration Ratios. The relative concentrations of diacid chloride and diamine vary widely between authors. Ishizaka and colleagues (95) for example reacted 0.2M piperazine in 0.225 M sodium carbonate with 0.04M terephthaloyl chloride, and 0.4M 1,6 hexamethylenediamine with 0.08M sebacoyl chloride. Luzzi and co-workers (99) used 0.58M 1,6 hexamethylene-diamine with 6.7×10^{-3}M sebacoyl chloride and Koishi and others (101) 0.4M piperazine or 1,6 hexamethylene-diamine with 0.04M phthaloyl chloride.

The phase volumes also vary considerably from author to author. In the preparation of microcapsules by Aisina and others (94) the volume of the internal aqueous phase was 1.5ml and that of the external organic phase was 15ml.

Luzzi and co-workers (99) however used an internal phase volume of 50ml and an external phase volume of 330ml.

5. Inclusion of Additives. In the original method of microcapsule preparation Chang and others (29) encapsulated haemoglobin. It has been suggested that the encapsulated haemoglobin may have the effect of maintaining the internal pressure of the microcapsules and may also directly crosslink the linear strands of the nylon polymer, thereby strengthening the membrane (94, 102). Other workers therefore, who have encapsulated haemolysate and other enzymes containing reactive amine groups may have inadvertently included a crosslinking agent in their formulation. A crosslinking agent which has been deliberately included in the preparation of microcapsules to strengthen the membrane and act as a capsule 'filler' is polyethyleneimine (94, 103). Polyethyleneimine was selected by Aisina and co-workers (94) as it was not washed out of the capsules, it contained reactive amino groups and was therefore capable of crosslinking and because it was not hydrolysed by the proteolytic enzymes which were encapsulated. Vandegaer and Wayne (104) suggested the use of the bifunctional monomers trimesoyl trichloride and 1,3,5 benzenetriamine.trihydrochloride for crosslinking the linear strands forming the polyamide microcapsule membranes. They reported that the extent of the crosslinkage governed the strength, rigidity and porosity of the capsule membrane. Degennaro (96) used diethylenetriamine and triethylenetetramine as crosslinking agents in the preparation of nylon 6.10 microcapsules.

The crosslinking agents were included in order to slow the release of sodium pentobarbital from the microcapsules. Other microcapsule 'fillers' which have been used include bovine serum albumin and polyvinylpyrrolidone (105). The bovine serum albumin possesses reactive amino groups and is therefore capable of crosslinking. Polyvinyl pyrrolidone was also used by Takamura and co-workers (100) to increase the viscosity of the aqueous phase in order to alter the permeability of the microcapsules to electrolytes. Luzzi and others (99) used 2% methyl cellulose solution for this purpose. Jenkins and Florence (93) included polyethylene glycol 400 (PEG 400) in their formulation. By adjusting the relative amounts of PEG 400 and buffer solution they were able to obtain an aqueous phase containing the drug to be encapsulated, pericyazine embonate, either in solution or in suspension form. However, the microcapsules prepared by these workers had a continuous structure and did not possess a thin polymer membrane surrounding a liquid core (106). In an attempt to improve the method for the microencapsulation of pharmaceuticals by polyamides McGinity (107) included formalised gelatin in the core of the microcapsules to slow the release of drug. Microcapsules were prepared containing gelatin in the aqueous solution and after preparation the gelatin was hardened by adding formaldehyde to the organic phase. This had a slight effect on delaying the release of sodium sulphathiazole from the microcapsules. McGinity and others (90) also prepared nylon microcapsules containing calcium sulphate

and calcium alginate. Calcium sulphate microcapsules were prepared by adding calcium sulphate hemihydrate to the aqueous phase. In the case of the calcium alginate microcapsules sodium alginate was included in the initial aqueous phase and after capsule formation saturated calcium chloride solution was added to the reaction mixtures. A number of drugs were successfully encapsulated using this technique such as morphine sulphate and diphenhydramine hydrochloride. No information on their properties however was given.

44' diamino stilbene 22' disulphonic acid (DASSA) was included in the preparation of polyphthalamide microcapsules by Koishi and others (101) so that negative charges were incorporated on the surface of the microcapsules thereby preventing aggregation.

6. Emulsion Formation. The method of emulsion formation and stirring time also differs between authors. Shigeri and others (89) and Koishi and others (87) both report the use of a "Chemistirrer" stirring at 620 rpm with an emulsification time of 5 minutes. Degennaro (108) used a "Brookfield Counter Rotating Stirrer" for 15 seconds to form the emulsion. Emulsion formation was effected by Luzzi and colleagues (99) using a "Waring blender" for 30 seconds at "low speed". Depending on the droplet size required Jenkins and Florence (93) formed the emulsion either by stirring, by using a hand homogeniser or by using an ultrasonic homogeniser. They also prepared microcapsules by a method based on Nawab and Mason's

method of emulsion formation (109). Using this method the buffered aqueous diamine solution was dispersed as fine droplets by an electrical potential and sprayed directly into a stirred organic solvent containing the dichloride. This method has the advantage that the diffusing diamine is not removed from the proposed polymerisation site prior to the addition of the diacid chloride, as occurs with conventional stirred systems.

7. Temperature. In the majority of reports of microcapsule preparation in the literature the reaction has been carried out surrounded by ice (87, 92). Microcapsules have also been prepared at -10°C and 30°C (89).

8. Microcapsule Recovery. A wide variety of techniques for the transference of microcapsules to an aqueous phase have been reported in the literature. Many of these techniques are closely related to the original method described by Chang and others (29) and involve washing the microcapsules with various concentrations and volumes of Tween 20 solution, centrifuging at different speeds and resuspending in either sodium chloride solution or Tween 20 solution (87, 89, 92, 97). The Tween is used to prevent the microcapsules aggregating.

The polysorbate molecules adsorb onto the polyamide molecules thereby making the membrane hydrophilic. Mori and colleagues (110) collected microcapsules either by filtration or centrifugation. They were then washed with ethanol to remove any residual organic reagents which might be adhering to the membrane and finally washed

with water to remove the ethanol. This has the advantage of avoiding the use of surface active agents which may be difficult to remove. The microcapsules are also not subjected to a hypertonic medium causing crenation. Luzzi and others (99) recovered their microcapsules by either a spray drying process or by flash evaporation. In the case of the spray drying process the microcapsule slurry formed after removal from the organic phase was diluted with chloroform and spray dried. The internal temperature of the drier was maintained at 125°C. The collected powder was placed in a vacuum oven at 35°C for 10-12 hours to remove residual solvent and moisture. In the flash evaporation process the wet slurry was placed in a flash evaporator at 35°C for 24 hours. Degennaro (108) also reported the use of spray drying and flash evaporation for the recovery of formed microcapsules.

9. Modifications to the Microcapsule Wall Structure. Lim and Moss (111) reported a "two step room temperature interfacial polycondensation process". Microcapsules were initially prepared by a method similar to that of the original (29) using various diamines with either sebacoyl or terephthaloyl chloride. The surfactant was Span 85 and the organic phase consisted of 1 part chloroform and 4 parts cyclohexane. Three to four minutes after the addition of the diacid chloride the reaction was stopped by separating the microcapsules from the continuous phase using centrifugation. The separated microcapsules were then resuspended in a volume of pure cyclohexane equal

to the volume of the mixed solvent used in the first reaction. Another portion of the diacid chloride was added and the second reaction was allowed to continue for 3 minutes. Separation and transfer of microcapsules from organic to aqueous phase were accomplished by washing with 50% Tween 20 solution followed by saline. In the first reaction the diamine had a reasonably high affinity for the organic phase and therefore a relatively thick polymer network was produced around the aqueous droplets. By removing the formed microcapsules from the reaction mixture and immersing them in cyclohexane the affinity of the diamine for the organic phase was decreased. Therefore on addition of further diacid chloride polymerisation with any unreacted diamine will take place within the voids of the already formed network.

The converse situation has also been reported (112). In this case after recovering the microcapsules from the reaction mixture they were resuspended in a solvent for which the diamine had a higher affinity before the addition of a further volume of diacid chloride. Thus during the second stage of the process additional quantities of the diamine were drawn through the initially formed membranes. On the addition of the diacid chloride the two monomers polyermise on the organic side of the original membrane producing thicker walls. In both cases the authors have suggested these methods may be used to control the upper limit of permeability of the microcapsules.

Another variation of the original method has been to prepare microcapsules where the wall consists of a copolymer. For example, an organic liquid pesticide has been encapsulated in a polyamide-polyurea skin (104). The organic phase contained the pesticide, sebacoyl chloride and polymethylene polyphenylisocyanate and the external aqueous phase consisted of polyethylene glycol, piperazine, water and sodium hydroxide. In another example of a polyurea polyamide skin the aqueous phase contained ethylenediamine and diethylenetriamine and the organic phase contained toluene 2,4-diisocyanate and trimesoyl trichloride (104).

Another modification involves the preparation of microcapsules with multiple walls (105). Concentric multi-wall capsules in which each wall is separated by a layer of liquid were prepared as follows. Large droplets 1-10mm of a diacid chloride solution in 'mineral spirits' were introduced into a second solution containing diethylenetriamine in water. The suspension was allowed to stand for 2 minutes during which time a polymer shell formed around each drop. The capsules were carefully removed allowed to drain and immersed in a diacid chloride solution. A second interfacial film of the same polymer formed around the original capsule but was separated from the original shell by a thin layer of aqueous triamine solution. These double walled capsules were carefully removed, drained and re-immersed in the aqueous triamine solution to form a third film of polymer separated from

the second film by a thin layer of 'mineral spirits' solvent. Alternate dipping was continued until six distinct concentric shells each separated alternately by aqueous and solvent layers were formed about the original drop.

10. Preparation of Oil containing Microcapsules. Oil containing microcapsules have also been prepared by interfacial polycondensation of a diacid and a diamine. For example the pesticides ethyl and methyl parathion have been encapsulated by this process (34, 113). In this case it is necessary to form an oil in water emulsion with the diacid chloride dissolved in the disperse phase. The reaction will again occur on the organic side of the interface and the microcapsule membranes will 'grow' inwards. In the encapsulation of oil yellow dye (114) a solution of the dye in O-dichlorobenzene was prepared containing sebacoyl chloride and polyethylene glycol. This solution was emulsified in aqueous polyvinyl alcohol solution using a magnetic stirrer. On addition of one or more diamines to the aqueous phase a polyamide membrane was formed encapsulating the dye. The reaction was stopped after 30 minutes of agitation. The microcapsules were washed with water and fractionated.

1.5.3 Properties of Polyamide Microcapsules

1.5.3.1 Microcapsules Appearance

In all cases reported microcapsules formed were invariably spherical (29, 92, 93) although Chang and co-workers (29) noted that the smallest size range microcapsules in their study also contained a proportion of discoid or cup shape forms. Emulsion droplets which are normally spherical distort under shear (115) and this is possibly the reason for the appearance of these microcapsules. The appearance of the microcapsules prepared by Chang and others (29) also suggests that they are hollow. This is in contrast to the microcapsules prepared by Jenkins and Florence which have been shown by scanning electron microscopy to be of a solid nature (106). The structure of microcapsules prepared by other workers is difficult to assess due to the preparative technique used.

1.5.3.2 Microcapsule Size and Size Distribution

From consideration of the method of preparation of polyamide microcapsules it may be expected that the microcapsule size will be determined primarily by the size of the emulsion droplets formed during the microcapsule preparation. The size of the microcapsules formed therefore will be affected by those factors influencing emulsion formation for example, method of emulsification, stirring speed and surfactant concentration.

In the preparation of haemoglobin loaded nylon 6.10 microcapsules Chang and co-workers (29) noted the relationship between microcapsule diameter, stirring speed and surfactant concentration. Increasing the stirring speed resulted in microcapsules of a more

uniform size. This was also the effect observed on increasing the emulsifier concentration from 1% to 5% v/v. Further increasing the concentration of emulsifier had little effect. These workers also noted that microcapsules of a mean diameter of less than 20μm could not be prepared using a conventional stirring method. Smaller microcapsules however could be formed using a "Virtis 45" homogeniser. The role of the haemoglobin in forming the microcapsules in this study is unknown and therefore analysing the results is difficult.

To overcome this problem Koishi and others (87) prepared water containing polyphthalamide microcapsules under various conditions and determined their size distribution curves. The size of the microcapsules was again found to be strongly affected by the concentration of the emulsifier and the degree of mechanical agitation, as were the size distributions. Increasing the stirring speed and the Span 85 concentration both resulted in a reduction in microcapsule diameter. The size distribution curves became narrower with an increase in emulsifier concentration up to 5% v/v but above this concentration no appreciable change in their shape was observed. A marked change in the shape of the size distribution curve was also noted when the stirring speed was raised in the absence of surface active agent, the distributions again becoming much narrower. The effect of stirring almost disappeared however in the presence of Span 85 at a concentration of 5% v/v. Microcapsules were prepared using an organic phase consisting of chloroform: cyclohexane either 1:3 or 1:4. There was no difference observed between the

size distributions of the microcapsules prepared using the two different compositions. These effects of stirring rate and emulsifier concentration were observed by Mori and co-workers (110) in the preparation of polyamide microcapsules.

Ogawa and colleagues (88) examined the effect of pH of the aqueous phase on the formation of polyamide microcapsules. Although no data was given it was stated that microcapsules were generally larger if they were prepared at low pH than if prepared at high pH. This is possibly due to the effect of pH on the partition coefficient of the diamine (88). In the case of piperazine, decreasing the pH of the aqueous phase from approximately 12 to 9.5 resulted in an increase in the partition coefficient of the diamine for the aqueous phase. It also resulted in a decrease in the percentage of reacted piperazine. Thus it is possible that at low pH microcapsules with weak membranes coalesce upon breakage of the membrane to form larger microcapsules.

Varying the chemical structure of the intermediates has been shown by Shigeri and others (89) to influence the size distribution of microcapsules. Microcapsules were prepared from 1,6 hexamethylenediamine with sebacoyl chloride (A) and piperazine with phthaloyl chloride (B). The size distribution curve was narrower for type B microcapsules than type A and the mean diameters for type A and type B microcapsules were 5.90μm and 3.37μm respectively. As all the conditions for microcapsule preparation were the same the difference in the size distribution should, the authors

suggested, arise from the different polymerisation characteristics for each combination of monomers. Shiba and co-workers (92) prepared polyamide microcapsules containing bovine serum albumin using different combinations of the following monomers; 1,6 hexamethylenediamine or piperazine with sebacoyl chloride or p phthaloyl chloride. They observed that there was little difference in the size distribution curves of microcapsules prepared using different monomers.

Shigeri and others (89) also examined the effect of temperature during microcapsule preparation, the effect of monomer concentration and the effect of monofunctional monomer on microcapsule size. It was found that an increase in temperature from $-10^{o}C$ to $30^{o}C$ brought about a decrease in microcapsule size. The authors reported that since the effect of temperature on the size of the emulsion droplets was negligible the effect of temperature on microcapsule size must result from a change in the polymerisation characteristics of the monomers. An increase in the microcapsule size was observed as the monomer concentration decreased. Also in the presence of benzoyl chloride, a monofunctional acid chloride the microcapsule size was again seen to decrease. These findings were explained by correlating microcapsule size with the intrinsic viscosity of the polymer membrane in each case. This indicated to the authors that the membrane strength played an important role in determining microcapsule size. A method for the formation of large microcapsules which resulted in a broadening of the size distribution was

also postulated. It was proposed that at an early stage of the polycondensation reaction primary emulsion particles, with membranes of insufficient strength and coverage to prevent them from uniting, coalesce. This results in the formation of large secondary particles. The membrane on the secondary particle may then thicken to prevent further coalescence provided the polymerisation reaction is still continuing. If the polymerisation rate is too high coalescence will not occur initially.

This hypothesis seems to account for the process of formation of double microcapsules as observed by Shiba and others (92). Their explanation for this observation was that since the ratio of surface area to volume is larger for small liquid droplets than for large ones then the transfer of diamine from the aqueous phase to the organic phase is greater for the former than for the latter. Accumulation of the diamine at the interface should facilitate the formation of the polymer membrane. The small microcapsules having relatively thick membranes will be able, on collision, to penetrate the larger ones having very thin membranes. As the polymerisation is still occuring the membranes of the larger microcapsules will reform. It was also suggested that the formation of double microcapsules was closely related to the partition coefficient of the diamine. Indeed the double microcapsules were not found to form until the partition coefficient of the diamine reached a fairly high value.

1.5.3.3 Membrane Thickness

Few observations of membrane thickness have been reported for polyamide microcapsules prepared by interfacial polymerisation. Those thicknesses reported vary widely. On the basis of electron microscope studies Chang and co-workers (29) estimated the membrane thickness of nylon 6.10 microcapsules containing haemoglobin and of diameter 80μm, to be about 0.2μm. Lim and Moss (111) estimated the membrane thicknesses of capsules prepared by their method to vary between 0.07 and 0.09μm. Takamura and others (100) and Ishizaka and others (95) used the following mathematical formula to calculate the thickness of their membranes.

$$\Delta x = \frac{W.r}{d.A} \qquad \text{(equation 1.9)}$$

W is the theoretical weight of microcapsule membranes in unit volume of dispersion assuming the complete reaction of diamine and acid chloride.

r is the experimentally determined percent reacted diamine.

d is the density of the microcapsules membrane.

A is the total surface area of microcapsules in unit volume of dispersion.

Nylon 6.10 microcapsules were estimated to have a membrane thickness of 0.0224μm whereas the thickness of polyphthalamide microcapsules was 0.0133μm (100). No estimation of microcapsule diameter was given. It was shown by Ishizaka and others (95) that as the content of cyclohexane in the organic phase used in the capsule preparation increased, so the thickness of the membrane increased. The authors suggested this was because on decreasing the concentration of chloroform in the organic phase the partition coefficient of the diamine decreased.

As a result, less acid chloride reacted in the bulk of the continuous phase and the monomer was consumed in larger amounts at the interface resulting in a thicker membrane. They also suggested that the purity of the monomers had an effect on membrane thickness. Two batches of microcapsules which had been prepared were found to have a ten fold difference in the thickness of the membranes. The only difference between the two preparations was that in the preparation of microcapsule with the thicker membranes terephthaloyl chloride of a higher purity had been used.

1.5.3.4 Mechanical Properties

The flexibility of polyamide microcapsules was first noted by Chang and others (29). These workers observed that 100μm diameter haemoglobin loaded polyamide microcapsules folded when forced under hydrostatic pressure to flow slowly through a narrow portion of tapered glass tube. After passing through the tube the capsules gradually returned to their normal shape. In 1968 this work was extended by Jay and Edwards (116) who used a cell elastometer to measure the mechanical properties of nylon 6.10 microcapsules containing erythrocyte haemolysate. They showed that the nylon membrane had negligible resistance to bending but a very high resistance to stretching. Any stretch in the nylon was found to be almost irreversible. They estimated that the diameter of the smallest pipette through which a spherical microcapsule could pass without irreversible change was no smaller than 90% of the microcapsule diameter. The pressure required to do this was in the order of 10^5 dynes cm^{-2}.

These findings however have been questioned (117) since it is highly probable that haemoglobin molecules in the aqueous phase are chemically incorporated into the microcapsule membranes. Thus the workers did not measure the tension of membranes composed of nylon 6.10 alone.

The strength of microcapsule membranes has been noted by a number of authors. Shiba and co-workers (92) prepared microcapsules from a selection of diacid chlorides and diamines. They reported that the microcapsules prepared varied in mechanical resistance to centrifuging. Piperazine- phthaloyl chloride capsules had the greatest resistance. In the case of 1,6 hexamethylenediamine -phthaloyl dichloride microcapsules the strength of the membrane was affected by the concentration of bovine serum albumin incorporated into the aqueous phase. 1,6 hexamethylene-diamine -phthaloyl chloride microcapsules prepared in the absence of bovine serum albumin were mostly destroyed by centrifugation leaving only very small capsules. However, many large microcapsules could survive even after centrifugation when prepared in the presence of bovine serum albumin. This is probably due to incorporation of the protein into the microcapsule membrane thereby strengthening it.

The presence of strongly ionisable groups present in the membranes also affects the membrane strength as shown by Koishi and others (101). Sulphonated polyphthalamide microcapsules were prepared by including 4,4' diaminostilbene-2,2' disulphonic acid in the aqueous phase during microencapsulation. It was noted that the sulphonated polyphthalamide microcapsules were generally more resistant to centrifugation than the unsulphonated ones.

Migawaki and others (118) noted the mechanical strength of microcapsules prepared containing ovalbumin, haemoglobin and casein. They calculated that the weight fraction residues of the three amino acids lysine, arginine and histidine in ovalbumin, haemoglobin and casein were 23.3, 22.5 and 12.1% w/w respectively. This order corresponded to the order of the observed mechanical strength of the microcapsules. That is, the greater the percentage of amino acids present, the stronger the microcapsule membranes. This also suggests therefore that the proteins present in the aqueous phase are likely to participate in the polymerisation reaction.

1.5.3.5 Permeability

The permeability characteristics of nylon 6.10 haemolysate-containing microcapsules were measured by Chang and Poznansky (119) using a 'rapid mixing and sampling technique'. The microcapsules were of mean diameter 210.9μm and were equilibrated with 3×10^{-1}M sodium chloride before use. The test solutes were urea, creatinine, creatine, uric acid, glucose, sucrose, acetyl salicylic acid and tritriated water. The test solutions consisted of 0.5 m moles l^{-1} of the test solute containing the corresponding carbon 14 isotope dissolved in a 3×10^{-1}M sodium chloride solution, except in the case of the tritiated water. Equal volumes of the microcapsule suspension and test solutions were mixed. A sampling syringe was set to aspirate microcapsule-free aliquots at 1, 2, 4, 8, 16, 32 and 64 seconds. The aliquots were assayed for the radioactivity remaining. From the data obtained the permeability constants were calculated. These are shown in Table 1.2.

Table 1.2 Nylon microcapsules - Permeability Data (119)

Solute	Half-time for equilibration (sec)	Permeability constant ($P \times 10^{-4}$cm sec^{-1})
Urea	4.3	2.01
Creatinine	17.5	0.61
Uric acid	42.5	0.19
Creatine	16.6	0.75
Glucose	26.2	0.54
Sucrose	35.5	0.37
Acetylsalicylic acid	39.0	0.32
Tritiated Water	<1.0	-

The permeabilities of poly(hexanediamine terephthaloyl chloride) microcapsules reported by Lim and Moss (111) were of the same order as those reported by Chang and Poznansky (119). The solutes used included glycerol (molecular weight 92), glucose (molecular weight 180) and sucrose (molecular weight 342). The permeabilities of these solutes expressed as half equilibration times were 1.75, 4.9 and 10.6 seconds respectively.

Takamura and others (98, 100) used changes in electrical conductance to determine the permeability of polyphthalamide microcapsule membranes to electrolytes. The microcapsule dispersion was added to an electrolye solution in a conductance cell and the conductance monitored immediately after mixing. In all cases studied the distribution equilibrium of electrolyte between the inside and the outside of the microcapsules was established almost within 2 hours. The permeability constants were calculated using the equation $P = \frac{C_f V_m}{2.303 C_i A} \cdot S$ (equation 1.10)

where C_i and C_f are the initial and final electrolyte concentrations

V_m and A are the total volume and surface area of the microcapsules

S is the slope of the plot log $[(C_t-C_f)/(C_i-C_t)]$ against time. C_t is the concentration at time t.

The values of the permeability constants were found to be of the order of 10^{-8}cm sec^{-1} and independent of temperature. This apparent low permeation rate was attributed to the formation of a stable diffusion layer of the electrolytes in the interior of the microcapsules. The membrane material was shown to have an effect on permeability and the apparent

coefficients of sodium chloride and sodium sulphate were reported for a variety of polyamide membranes. The diffusion coefficients were greater for polyamides with polymethylene chains than with aromatic rings. This is probably due to the difference in polarity and degree of hydrogen bonding of the polymer chains formed by the two types of monomer. The effect of increasing the viscosity of the aqueous phase by the addition of polyvinylpyrrolidone was to decrease the permeability of the sodium chloride and sodium sulphate. This was interpreted as indicating that the diffusion of the electrolyes in the interior of the microcapsules was rate determining in the permeation process.

The permeability coefficients for sodium, potassium and rubidium hydroxides in polyphthalamide and nylon 6.10 microcapsules were measured by Ishizaka and others (95). The coefficients were estimated by determining the change in the hydroxyl ion concentration of the medium with time by means of a pH meter. Reported permeability coefficients for sodium hydroxide, potassium hydroxide and rubidium hydroxide were 1.82×10^{-11}, 4.91×10^{-11} and 1.46×10^{-12} cm sec^{-1} respectively. It was shown that on increasing the proportion of cyclohexane in the organic phase used in the preparation of the microcapsules, the membrane thickness increased and there was a corresponding decrease in the permeability. The permeability was also affected by the nature of the polymeric membranes, the permeability coefficient in polyphthalamide membranes being 30 to 350 times higher than in nylon 6.10. This is not in agreement with the findings of Takamura and colleagues (100) described above. A possible reason for this is the different conditions used in the capsule

preparation which alters the interaction between the solvent and the polymer. The authors suggested it was likely that ion permeation depended strongly on the interaction between water and the ions when they passed through the pores in the polymer membrane. In order to examine the water structure in and around the polymer membrane a differential scanning calorimeter was used. It was observed that on the addition of increasing concentrations of urea, which is believed to promote water structure formation, the potassium hydroxide permeability coefficient first increased and then decreased. The initial increase was interpreted as being due to the predominant effect of the increase in water structure which occurs on the addition of urea. The decrease was interpreted as being due to the decreasing cation potential gradient which also occurred on the addition of urea and became the predominant feature at high urea concentrations. From these findings it was concluded that the ion permeation was related to the water structure in and around the microcapsule membrane.

Miyawaki and co-workers (118) used an ammonium chloride tracer to determine the permeability of polyamide microcapsules. The microcapsules were placed into an ammonium chloride solution and allowed to equilibrate. The suspension was allowed to sediment and the supernatant removed. To the capsule layer was added a known amount of buffer and the release of ammonium chloride from the capsules to the external solution was measured using an ammonium electrode. The permeabilities were determined for microcapsules prepared containing aqueous solutions of ovalbumin at pH7.0 and 4.6, haemoglobin at pH7.0

and casein at pH7.0. The permeability coefficients were 0.608, 1.53, 2.65 and 2.46 x 10^{-3}cm min^{-1} respectively. The fact that the presence of these proteins influenced the permeability coefficient confirmed the finding of Shiba and others (120) that the protein participated in the copolymerisation reaction. The authors noted the discrepancy between their data and that of the previous workers and suggested this could be due to the following:

1. The method by which the data was obtained.

2. The point on the full release profile at which measurement was commenced.

3. The mean diameters of the microcapsules in each case.

According to Kondo and Koishi (121) the quantity of water bound to the capsule membranes increases in a very small capsule. From the work of Ishizaka and others (95) this will clearly influence the permeability and therefore permeability will be affected by size.

The rate of release of sulphathiazole sodium from nylon microcapsules and formalin treated nylon microcapsules containing gelatin was investigated by McGinity (107). The release characteristics of the two types of microcapsule were studied in 0.1N HCl and 0.1M acetate buffer pH 5.6. The dissolution apparatus consisted of a round bottom flask into which was placed a stirring blade. The stirrer was rotated at 100 rpm and the release of drug was measured

spectrophotometrically. The release of the drug into dilute acid from the nylon and the formalin treated nylon gelatin microcapsules was delayed. There was however little difference between the two formulae 25-35% being released in the first 2 minutes in both cases and 99% release occuring between approximately 30 and 60 minutes. A greater retardant effect was seen when examining the release into pH 5.6 acetate buffer 50% release occuring after approximately 40 minutes. Again however there was little difference between the two formulae. The difference between the data for the two release media possibly indicates that the presence of the acid was adversely affecting the membrane structure. However, the effect of the presence of 0.1N HCl on the release of sodium pentobarbital measured by Luzzi and co-workers (99) was to decrease the release rate when compared with the release into water. The effect of the acid may therefore be purely a pH effect. The release of the sodium pentobarbital from polyamide microcapsules was measured by a similar method to that of McGinity (107) but the suspensions were stirred at only 6 rpm. Microcapsules prepared by a flash evaporation process had much faster release rates than those which had been spray dried. This may have been due to the breakage of a portion of flash evaporated microcapsules during processing. These broken microcapsules would release their contents immediately resulting in an increase in the observed release rate. In both cases however 50% of the drug content was released within 15 to 30 minutes. Luzzi and co-workers also reported on the release from tabletted microcapsules. As expected the release was dependent upon tablet hardness. It was also dependent upon the release medium used.

Much smaller release rates were reported by Florence and Jenkins (122) for polyamide microcapsules containing pericyazine embonate and trifluoperazine embonate. The time taken for release of 50% trifluoperazine embonate was in the order of 4 hours. The discrepancy between this data and release rates measured by other workers may arise as a result of the apparent continuous structure of the microcapsules prepared by these authors (106).

The effect of change in the polymerisation reaction condition as the release from microcapsules was studied by Degennaro (108) and Naik (123). The latter encapsulated amaranth and used this to determine the effect on the release profile of the nature of the monomers, the nature of the surfactant and the concentration of the acid chloride. The time taken for 50% amaranth release varied in all cases between only 20 and 30 minutes. Phenylpropanolamine hydrochloride was also encapsulated and it was shown that the rate of release of the drug decreased with an increase in the concentration of polymer forming materials. This is probably to be expected, as an increase in the amount of monomers present will increase the thickness of the wall formed. The rate of release of phenylpropanolamine hydrochloride also decreased with an increase in the concentration of hydroxypropyl cellulose encapsulated along with the drug. This was probably due to the increased viscosity of the core which would arise on the encapsulation of increasing amounts of the cellulose. This increased viscosity would decrease the diffusion of the drug to the microcapsule wall.

Degennaro (108) used sodium pentobarbital as the core material and found that its release from polyamide microcapsules was affected by the choice of organic solvent, emulsification time, stirring speed, the relative ratios of aqueous and organic phases, the presence of crosslinking agents and the size of the microcapsules. It was found for example that an emulsification time of 5 seconds yielded microcapsules having a somewhat faster release rate than those produced using a 15 second emulsification period. A faster release rate was also obtained from those microcapsules which had been prepared using a slower rate of stirring. Alteration of the ratio of aqueous to organic phase produced microcapsules having different release rates. Those microcapsules prepared using an aqueous phase of 200ml and an organic phase of 500ml produced microcapsules having a faster release rate than when an aqueous phase of 100ml was used. It was also reported that there was an optimum ratio of diamine to acid chloride for the preparation of nylon 6.10 microcapsules. This was found to be 0.41 moles of diamine to 1 mole of acid chloride. The progressive addition of the crosslinking agents diethylenetriamine and triethylenetetramine produced microcapsules having increased rates of release. The increase followed no discernible pattern. It was suggested the presence of the crosslinking agents resulted in microcapsules with membranes of increased porosity, which therefore resulted in the increased release rate observed. The effect of microcapsule size on the release rate was also investigated. It was found that the smaller microcapsules had a faster release rate than the larger ones. This was in agreement of the findings of Kondo of Koishi (121) and Ishizaka and others (95). In this report typical time

intervals taken for 50% release of the core material varied between only 5 and 10 minutes.

1.5.4 Polyamide Microcapsules in Pharmacy and Medicine

The first suggested use for polyamide microcapsules was as artificial red blood cells (29). Encapsulation of haemolysate resulted in the preparation of microcapsules containing haemoglobin which maintained some enzymatic activity and the ability to combine with oxygen. These microcapsules however were unsatifactory as a model for red blood cells as they were too large in size and too rigid.

A further suggested use for these microcapsules is as artificial cells in an extracorporeal shunt system, or for use as an artificial kidney (119, 124, 125). In haemodialysis one of the toxins to be removed from the blood is urea. The urea does not readily adsorb onto the activated carbon which is the adsorbent normally used in haemodialysis. If urease is encapsulated with a polyamide shell it has been found that the urea readily permeates the microcapsule wall and is broken down into ammonia and carbon dioxide. These degradation products are readily adsorbed by the carbon. The advantage of using encapsulated enzyme is that it acts without coming into direct contact with protein and formed elements of the blood and therefore does not provoke an immunological reaction. The large surface area which can be generated using microencapsulation is also advantageous as it decreases the total volume necessary for haemodialysis. In order to decrease the possibility of a thrombosis caused by interaction between the

blood and the microcapsules in an extracorporeal shunt system. Chang and co-workers (126) reported on the preparation of non-thrombogenic microcapsules. These nylon microcapsules were prepared with heparin complexed membranes.

Other enzymes which have been encapsulated using this technique include catalase and L asparaginase. Encapsulated catalase in the form of a paste was applied topically to acatalasaemic mice to remove hydrogen peroxide in oral lesions (127). Two advantages were observed. First, the enzymes acted efficiently on the hydrogen peroxide without being absorbed and without producing any immunologic or hyposensitive reactions. Secondly, the microcapsule paste remained at the site of action longer than the catalase solutions. Encapsulated catalase has also been injected into acatalasaemic mice for the enzyme replacement in acatalasaemia (128). L asparaginase, an enzyme which suppresses the growth of certain tumours, was also microencapsulated by Chang (129). Parenteral injections of encapsulated L-asparaginase and free L-asparaginase were administered to mice with tumours. The comparative results showed that the microencapsulated form was more effective than the free solution in suppressing the growth of the induced lymphosarcoma. The reason for this was thought to be the increased duration for which the microencapsulated enzyme remained in the peritoneum.

Reports in the literature of drugs which have been microencapsulated using the interfacial polymerisation technique are few. Such drugs include pen tobarbital (99)

sulphathiazole sodium (107) and pericyazine (106). The reason for encapsulating those drugs cited above was to control the release of the active ingredient from the microcapsule core. The release of these microencapsulated drugs is discussed in Section 1.5.3.5.

MATERIALS AND METHODS

2.1 Materials

2.1.1 Drugs

Pilocarpine nitrate was used as obtained from MacFarlan Smith Ltd., Edinburgh. The purity of the pilocarpine nitrate was assessed by TLC and by melting point determination as discussed in Section 2.4.1.1.

Prednisolone sodium phosphate (Becpharm Ltd.) was also used without further purification.

Ethyl-4-aminobenzoate (BDH Chemicals Ltd.) was recrystallised from 50% ethanol before use.

Tritiated pilocarpine was obtained from New England Nuclear and from the same source as a gift from Smith and Nephew Ltd.

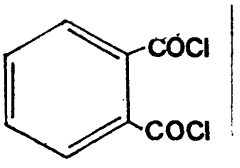
2.1.2 Reagents used in Microcapsule Preparation

Sebacoyl chloride (BDH Chemicals Ltd.) was re-distilled under vacuum at 0.2mm Hg and 106°C before use.

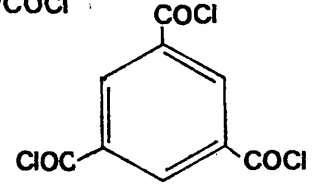
$$Cl\ C\ O\ (CH_2)_8\ COCl$$

The following monomers were used without further purification:

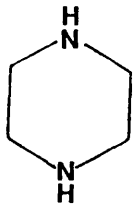
phthaloyl chloride (BDH Chemicals Ltd.)



1, 3, 5 benzene tricarboxylic acid chloride (Aldrich Chemical Company)



1,6 hexamethylenediamine (BDH Chemicals Ltd.) $NH_2(CH_2)_6NH_2$

piperazine hydrate  $\cdot 6H_2O$

diethylenetriamine (Aldrich Chemical Company) $H_2NCH_2CH_2NHCH_2CH_2NH_2$

Gelatin was obtained from BDH Chemicals Ltd.

Bovine Serum Albumin and polyethyleneimine (molecular weight range 50,000 to 60,000) were obtained from Sigma Chemical Company.

Cyclohexane and chloroform (BDH Chemicals Ltd.) were AnalaR grade.

Span 85 (sorbitan trioleate) and Tween 20 (polyoxyethylene 20 sorbitan monolaurate) were obtained from Atlas Chemical Company.

2.1.3 Chemicals used for preparing Liquid Scintillation Cocktail

Xylene obtained from BDH Chemicals Ltd. was Scintillation Grade.

PPO (2,5 diphenyloxazole), dimethyl POPOP (1,4-bis[2(4-methyl-5-phenyloxazolyl)] benzene) and Triton X114 (octyl phenoxy polyethoxyethanol) were obtained from Sigma Chemical Company.

2.1.4 Miscellaneous Materials

Albumin fluorescin isothiocyanate and fluorescin isothiocyanate dextran were obtained from Sigma Chemical Company.

The kit for labelling human albumin with ^{99M}Tc was obtained from International CIS France. ^{99M}Tc was supplied by Queens Medical Centre, Nottingham. The albumin was labelled by injecting a known volume of ^{99M}Tc sodium pentechnetate solution of known activity into a vial containing 10mg human albumin. The vial was shaken, allowed to stand for 20 minutes and used immediately.

Non oriented nylon 6 film was Carran grade 77c.

N-1-naphthyl ethylenediamine dihydrochloride was obtained from BDH Chemicals Ltd.

All other chemicals, solvents and reagents were of AnalaR quality wherever possible.

2.2 Instrumentation/Equipment

Liquid Scintillation Counting System. Liquid Scintillation Counting was carried out using an LKB Rakbeta 1215 liquid scintillation counter (Wallac Oy Finland). Samples to be assayed were dispensed into 5ml capacity polyethylene mini vials (LKB) contained within 22ml capacity glass vials (LKB). Sample volumes were measured using a replicating pipette (Oxford Ltd.) with delivery volumes of 0.2, 0.5 and 1.0ml fitted with plastic disposable tips.

HPLC System. The HPLC system consisted of a Constametric 11G pump (Laboratory Data Control, Staffs.) connected to a Pye-Unicam Type LC3UV variable wavelength detector and Tekman TE 200 flat bed chart recorder. 10cm stainless steel columns (0.5mm I.D.) were packed with Hypersil 5 C_{18} (Shandon Southern) or Partisil 10 S AX (Whatman). Samples were injected onto the column using a Rheodyne 20µl loop valve.

Size Analysis. Size analysis was undertaken using a Coulter Counter model ZB (Coulter Electronics Ltd.) and a Particle Size Micrometer and Analyser Type 526 (Fleming Instruments Ltd.).

Microscopy. A Carl Zeiss Jena microscope with camera attachment was used for microscopy work and for photographing prepared slides. Electron microscopy studies were undertaken using a JEOL 100 CX Transmission Electron Microscope with ASID Attachment (scanning) and Energy Dispersible X-Ray Detector (JEOL UK Ltd. London) (EDAX Illinois) as appropriate.

pH Meter. Measurement of pH was carried out using a Pye Unicam model 291 pH meter in conjunction with a Pye Unicam Ingold 401 glass silver-silver chloride combined electrode. Scale range 0 - 14 pH units.

Spectrophotometer. Absorbance measurements were determined using a Unicam SP 500 UV and visible spectrophotometer. Range 190-700nm.

Waterbath. A Grant shaking water immersion bath type SS 30 was used. This was set at $32^{o}C \pm 0.1^{o}$ throughout.

Balance. An Oertling model R20 single pan analytical balance was used for weights in excess of 0.1g. Below 0.1g a Stanton Instruments Unimatic HCL 5D single pan analytical balance was used.

Rotary Evaporator. The rotary evaporator used was a Büchi Rotavapor-R.

Centrifuge. An MSE bench centrifuge was used throughout.

Overhead Stirrer Motor. The overhead stirrer motor was a Citenco type DTS 7333. The stirring speed was varied by means of a Variac rheostat type VSHM connected to the motor. A stroboscopic light was used to determine the speed of stirring.

Gamma Camera. The gamma camera used was a Maxi Camera 11 obtained from The International General Electrics Company of New York. It was fitted with a pinhole collimator and spacer.

Vernier Microscope. The vernier microscope was obtained from The Precision Tool and Instrument Company, Surrey.

Melting Point Apparatus. The melting point apparatus was supplied by Gallenkamp Ltd.

TLC Plates. TLC plates were pre-coated and obtained from Anachem Ltd. The adsorbent was silica gel G of 250μm thickness.

Animal Model. The animal model used throughout this work was male New Zealand White rabbits of approximate body weight 3.0 Kg.

Analytical Glassware. Analytical glassware was Grade A.

2.3 General Methods

pH Measurement

Solutions under test and standard buffers were equilibrated to 25°C ± 0.1°C prior to pH measurement or pH meter standardisation. pH meters were calibrated before use with two standard buffers:- borax (pH 9.180 at 25°C) and phosphate (pH 7.413 at 25°C). Standard buffers were prepared according to the method of Perrin and Dempsey (130). When not in use the buffers were stored in stoppered flasks in a refrigerator and discarded every four weeks.

Temperature Measurement

Throughout this study all temperature measurements were made with reference to a calibrated mercury in glass thermometer graduated at intervals of 0.1°C.

Spectrophotometric Determination

1cm path length quartz cuvettes were used throughout this work. The solvent systems used for preparing the solutions to be measured were placed in the reference cell in all cases.

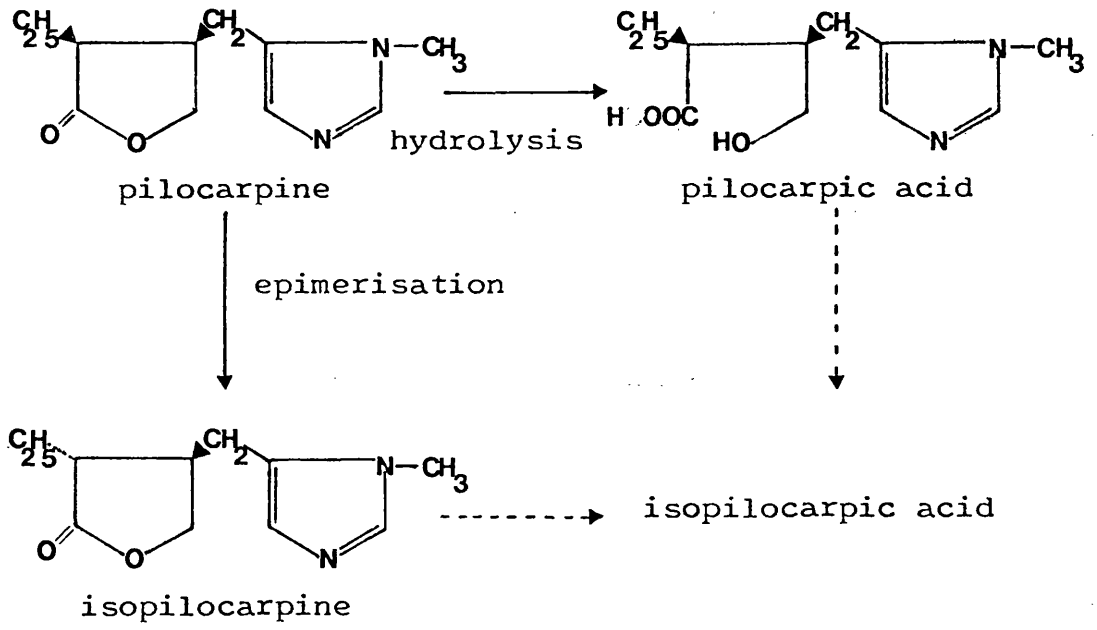
2.4 Analytical Methods

2.4.1 Pilocarpine Nitrate

2.4.1.1 Purity of pilocarpine nitrate

It has been reported that pilocarpine hydrolyses to form pilocarpic acid or epimerises to form isopilocarpine. The pilocarpic acid and isopilocarpine may then degrade to form isopilocarpic acid (131) as shown below.

Figure 2.1 The Degradation of Pilocarpine



Two methods were used to detect the presence of degradation products in the pilocarpine nitrate used in this study, namely melting point determination and thin layer chromatography (TLC).

Melting Point Determination. Purified pilocarpine nitrate was obtained by recrystallisation of the drug from ethanol. After recrystallisation the pilocarpine nitrate was dried under vacuum over phosphorous pentoxide at room temperature until constant weight was achieved. The melting points of both the original

material and the recrystallised material ranged between 170°C and 170.5°C.

TLC Method. 10µl samples of a 3.69×10^{-2}M aqueous solution of pilocarpine nitrate were spotted onto silica gel GF plates of 250µm thickness. The running solvent was equivolume methanol and chloroform (132) and the spots were visualised with iodine vapour. Standard solutions of purified pilocarpine nitrate recrystallised from ethanol, degraded pilocarpine nitrate and isopilocarpine base were spotted on the same plate. The R_f values are given in Table 2.1. The degraded pilocarpine nitrate was obtained by heating with 0.1M sodium hydroxide at 90°C for 1 hour.

The pilocarpine nitrate showed no degradation detectable by TLC throughout the study and was therefore used without further purification.

Table 2.1 R_f Values for TLC of Pilocarpine Nitrate and its Degradation Products

Compound	R_f determination 1	R_f determination 2
Pilocarpine nitrate	0.70	0.70
Pilocarpine nitrate (purified)	0.68	0.70
Isopilocarpine base	0.71	0.71
Degraded pilocarpine nitrate (assumed to be pilocarpic acid)	0.22	0.23

2.4.1.2 Assay of Pilocarpine Nitrate by Liquid Scintillation Counting

Introduction

A beta-emitting isotope such as tritium (^{3}H) or carbon 14 (^{14}C) decays with the emission of a detectable, negative, beta particle which carries a quantum of energy within a range characteristic of a given isotope. In the environment of a liquid scintillator the kinetic energy of these nuclear particles is converted into light photons which may then be counted. The liquid scintillator is composed of a solvent, a number of fluors and several additives. The function of the solvent molecules is to convert the kinetic energy of the nuclear emissions into molecular excitation energy and to transfer this energy to a fluor molecule. The fluor molecule re-emits the energy as light photons which in a scintillation counter are converted to electrical pulses. The amplitude of each pulse is directly proportional to the energy of the initiating beta particle and the number of pulses is directly related to the number of decay events.

Interference or competition with the orderly progression of energy transfer from the nuclear particle to the fluor reduces the fluorescence and thereby the detected pulses. This phenomenon is known as quenching and may occur due to the presence of additives or to the specimen itself. If each sample is not quenched to the same degree, for example, as a result of the presence of impurities, it is necessary to determine the degree of quenching and correct for it. One method of doing this is by External Standards Ratio. In this method a gamma emitting isotope is positioned adjacent to the sample vial. The gamma

radiation induces Compton electrons and hence scintillation in the sample by their interaction with the solvent molecules. This results in the instrument 'seeing' a pulse height spectrum representative of the external standard source. This Compton spectrum will be affected by quenching materials in a similar way to the spectrum produced from the sample's beta emitting source. Hence, by calibrating with quench standards of known disintegrations per minute (dpm), observing the measured counts per minute (cpm) and noting the calculated external standard ratio numbers, correlation curves of counting efficiency and external standard ratio can be obtained, where efficiency is the ratio of cpm to dpm. The disintegrations per minute of any unknown sample may then be calculated from the observed counts per minute and the efficiency.

Pilocarpine Nitrate Assay

Unless otherwise stated pilocarpine nitrate was assayed using a liquid scintillation counting technique. A known activity of tritiated pilocarpine was incorporated into the stock solution of pilocarpine nitrate of known concentration used for each experiment. The activity of the tritium in subsequent dilutions of this stock solution was then determined by the following method:

Five 200µl samples were removed from each solution to be assayed using a replicating pipette. Each sample was placed into a 5ml capacity mini vial and 4.5ml of scintillation cocktail added and shaken. The scintillation cocktail, which was suitable for incorporating aqueous vehicles, consisted of the following:

Xylene	1500ml
Triton X114	500ml
Dimethyl POPOP	0.4g
PPO	6.0g

Samples were counted employing the appropriate quench curve to determine the disintegrations per minute. Each sample was counted for 10,000 counts or 10 minutes whichever was the shorter, with an external standard time of 30 seconds. Background count was subtracted from the mean of the five measured disintegrations per minute before any calculations were made. The value of background count was generally found to be 60 dpm ± 5 dpm. The highest and lowest background counts observed were approximately 80 dpm and 40 dpm respectively.

Reproducibility of Sampling

To assess the reproducibility of sampling a volume of 200µl using a replicating pipette repeated measurements of the weight of nominal 200µl samples of distilled water were made. Table 2.2 gives the delivery weights for two sets of ten samples. From the data in Table 2.2 it was considered that the accuracy and precision of delivery of 200µl by the replicating pipettes was satisfactory.

Quench Curve Construction

Quench curves were constructed using the external standards ratio method for each of the following solvent systems. These solvent systems were sampled and assayed for tritiated pilocarpine at various stages of the work.

	Delivery Weight (g)	Mean Delivery Weight (g)	Standard Deviation	Coefficient of Variation
Determination 1	0.2006 0.2003 0.1972 0.1942 0.2052 0.2028 0.2024 0.2022 0.2015	0.2007	0.0033	± 1.6%
Determination 2	0.1963 0.2026 0.2027 0.2031 0.2018 0.2030 0.1993 0.2044 0.2040 0.2031	0.2020	0.0024	± 1.2%

TABLE 2.2 MEAN DELIVERY WEIGHTS OF A NOMINAL 200µl VOLUME DELIVERED BY A REPLICATING PIPETTE.

Chloroform

Chloroform: cyclohexane 1:5 containing 1% v/v Span 85

Acetone

Phosphate buffer pH 7.4

5% v/v aqueous Tween 20 solution

Into each of six mini vials was placed a capsule of known activity (LKB cat. n^{o} 1210 - 120 dpm = 101,100 [0.09μCi] corrected for decay) and 4.5ml scintillation cocktail. To each sample 200μl distilled water was added. Vial 1 was capped. To vials 2, 3, 4, 5 and 6 was added 5, 10, 20, 40 and 100μl chloroform respectively. The samples were loaded into the LKB 1215 liquid scintillation counter which had been programmed to count and automatically construct a quench curve which is shown in Figure 2.2. The process was repeated for the four remaining solvent systems using volumes 0, 20, 40, 100 and 200μl. All quench curves were found to lie on the same line as that for chloroform and this curve was therefore stored in the instrument's memory so that disintegrations per minute for all subsequent samples were calculated automatically (Figure 2.2).

Purity of Tritiated Pilocarpine

The purity of tritiated pilocarpine was determined by two methods.

i) The TLC method outlined in Section 2.4.1.1 was used to assess the degradation of the tritiated pilocarpine. 10μl samples of a 3.69×10^{-2}M aqueous solution of pilocarpine nitrate also containing tritiated pilocarpine

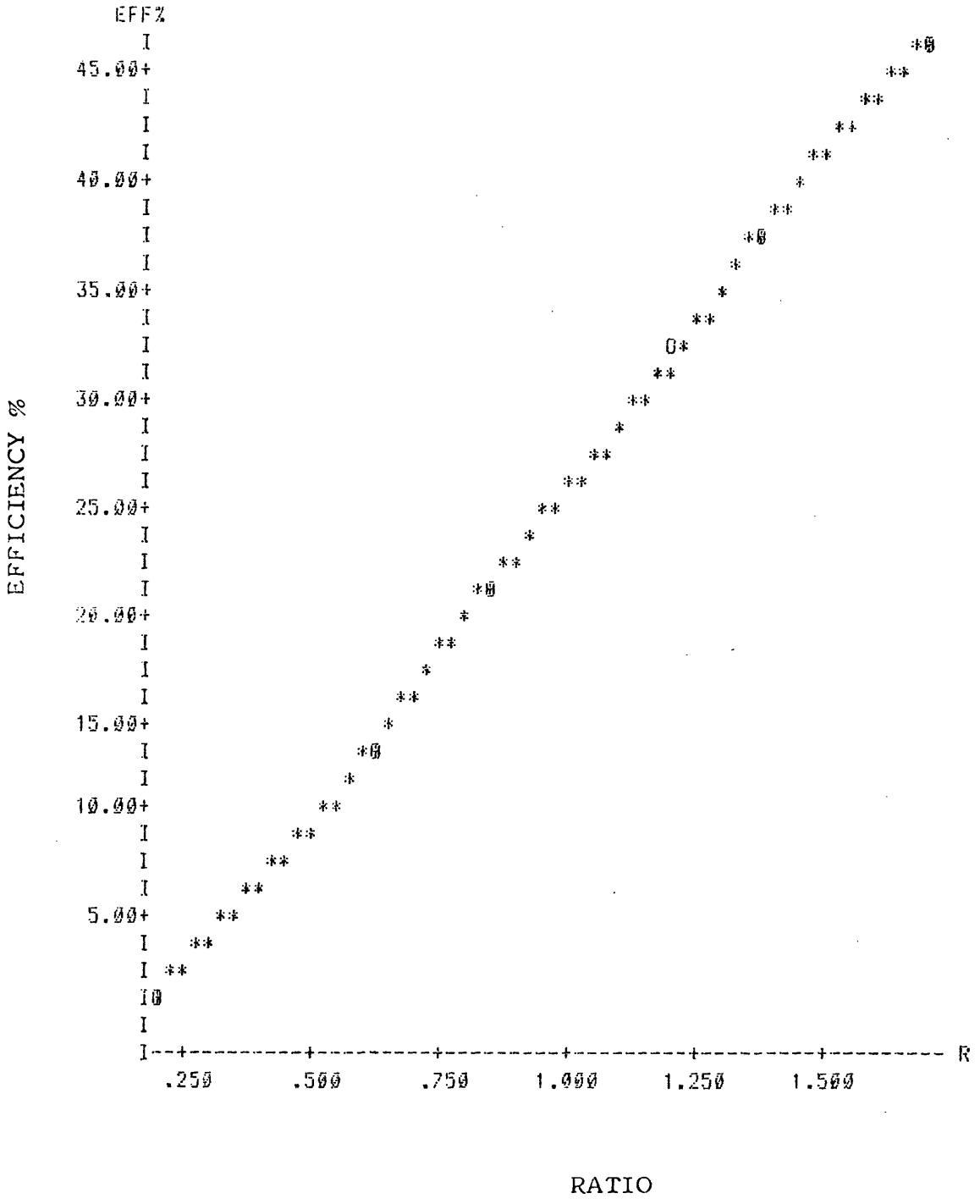


FIGURE 2.2 QUENCH CURVE FOR TRITIUM DETERMINED BY LKB 1215 LIQUID SCINTILLATION COUNTER.

were spotted onto silica gel TLC plates. The unlabelled material was present as a carrier for the labelled drug. After elution the TLC plates were scraped at 1cm intervals and each scraping placed in a 5ml capacity mini vial together with 200µl distilled water and 4.5ml scintillation cocktail. These were then assayed for tritium. The results are shown in Figure 2.3 as a histogram. The tritiated pilocarpine showed no degradation products, there being only one region of activity found on the TLC plate. This corresponded to the R_f value obtained for unlabelled pilocarpine nitrate when visualised by Iodine vapour (see Section 2.4.1.1) on a TLC plate which was eluted at the same time as that used for determination of tritiated pilocarpine nitrate purity.

ii) It is well known that tritiated compounds may exchange the tritium label with water. In order to assess the degree of exchange of tritium with water for pilocarpine stock solutions, 200µl of tritiated pilocarpine solution was pipetted into each of ten mini vials. To five of these was immediately added 4.5ml scintillation cocktail. The remaining five were evaporated to dryness before adding 200µl distilled water and 4.5ml scintillation cocktail. The ten vials were assayed for tritium. Table 2.3 gives the percent of tritium remaining attached to the pilocarpine molecule. It is apparent that there was a rapid exchange of tritium with water. Approximately 5% exchange occurred within 3 days and consequently stock solutions were evaporated to dryness and reconstituted immediately prior to use.

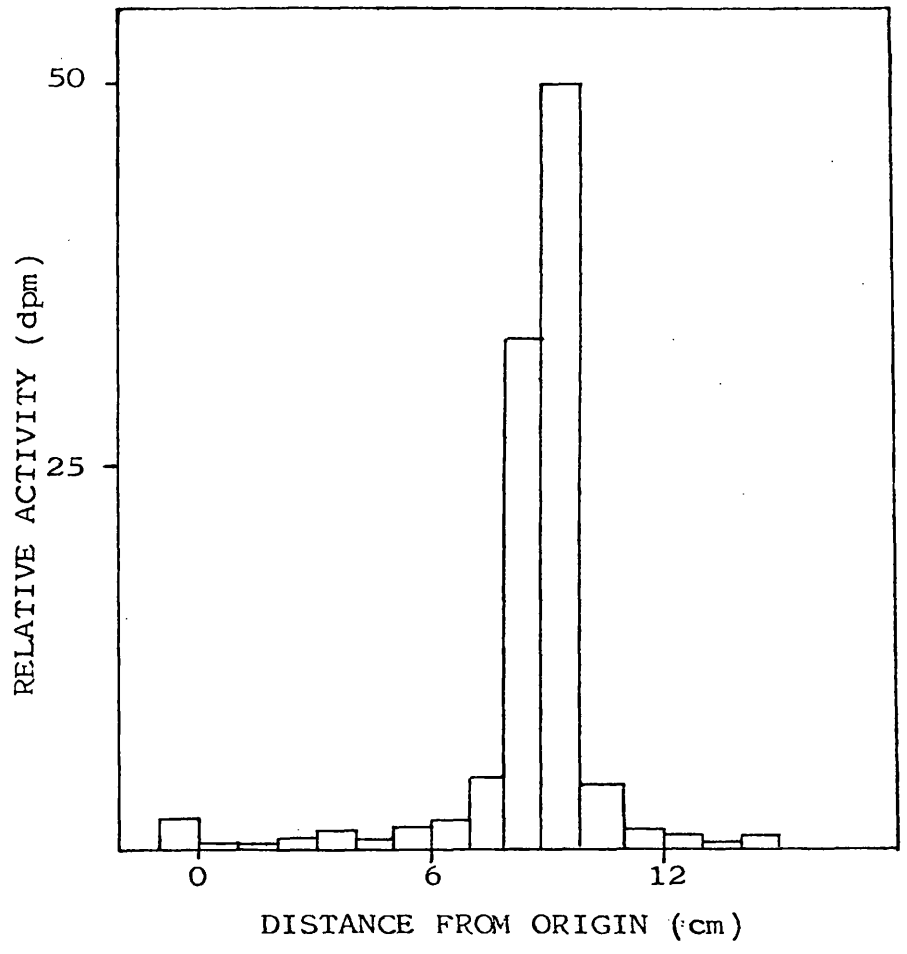


FIGURE 2.3 DISTRIBUTION OF TRITIATED PILOCARPINE ON TLC PLATE AFTER ELUTION.

Table 2.3 Tritium Exchange Between Pilocarpine and Water

Time	Percent Activity Remaining after Evaporation	Percent Exchange with Water
-	99.2	0.8
1 day	94.3	5.7
3 days	94.7	5.3
6 days	93.6	6.4
13 days	90.6	9.4
4 weeks	85.1	14.9

Reproducibility of Liquid Scintillation Counting

The reproducibility of the liquid scintillation counting technique was determined by preparing stock solutions of pilocarpine nitrate containing a known activity of tritiated pilocarpine. Five 200µl samples were removed from each solution for counting. Table 2.4 shows the disintegrations per minute obtained at three different levels of count. The coefficients of variation obtained were in all cases approximately ± 1.5%. In assessing the reproducibility of delivery of 200µl sample volumes, the coefficient of variation was found to be approximately ± 1.4% (Table 2.2) and therefore the inaccuracies involved in the liquid scintillation counting assay possibly arise from sampling error. The values obtained for the reproducibility of the liquid scintillation counting technique were considered satisfactory.

Calibration Curve for Pilocarpine Nitrate

In order to demonstrate that there was a linear relationship between relative count and relative concentration for a given series of pilocarpine nitrate solutions, a stock solution of

	Disintegrations per Minute	Mean Disintegrations per Minute	Standard Deviation	Coefficient of Variation
Determination 1	2756.3 2677.9 2761.1 2771.3 2779.3	2749.2	40.8	± 1.5%
Determination 2	14002.5 13834.6 14395.8 14295.6 14075.8	14120.9	225.9	± 1.6%
Determination 3	70021.6 71397.5 73004.8 71146.1 71037.8	71321.6	1077.1	± 1.5%

TABLE 2.4 REPRODUCIBILITY OF LIQUID SCINTILLATION COUNTING.

known drug concentration containing tritiated pilocarpine was prepared. Successive dilutions from this stock solution were made and the resulting solutions assayed for tritium. The results are given in Figure 2.4. Values for the slope and intercept of the calibration curve were calculated using least squares regression analysis (Appendix 1) and are given in Table 2.5. Reproducing each initial activity exactly was not possible and therefore only one set of representative data is given. The data show a high correlation coefficient and the intercept passes through zero within one standard deviation. It is apparent therefore that the activity of the tritiated pilocarpine is directly proportional to the concentration of pilocarpine nitrate present. This was therefore considered a satisfactory method for determining pilocarpine nitrate concentration provided that the activity of a standard solution of known pilocarpine nitrate concentration is assayed for each set of experiments. Subsequent data within that experiment may then be related to this single point reference standard.

Incorporation of Tritiated Pilocarpine - Effect on Concentration

Under all circumstances in which pilocarpine nitrate was assayed by a liquid scintillation counting technique tritiated pilocarpine was added to a solution of unlabelled drug of previously determined concentration. This addition therefore altered the concentration and an example of the calculation for correcting the total mass present is given below:

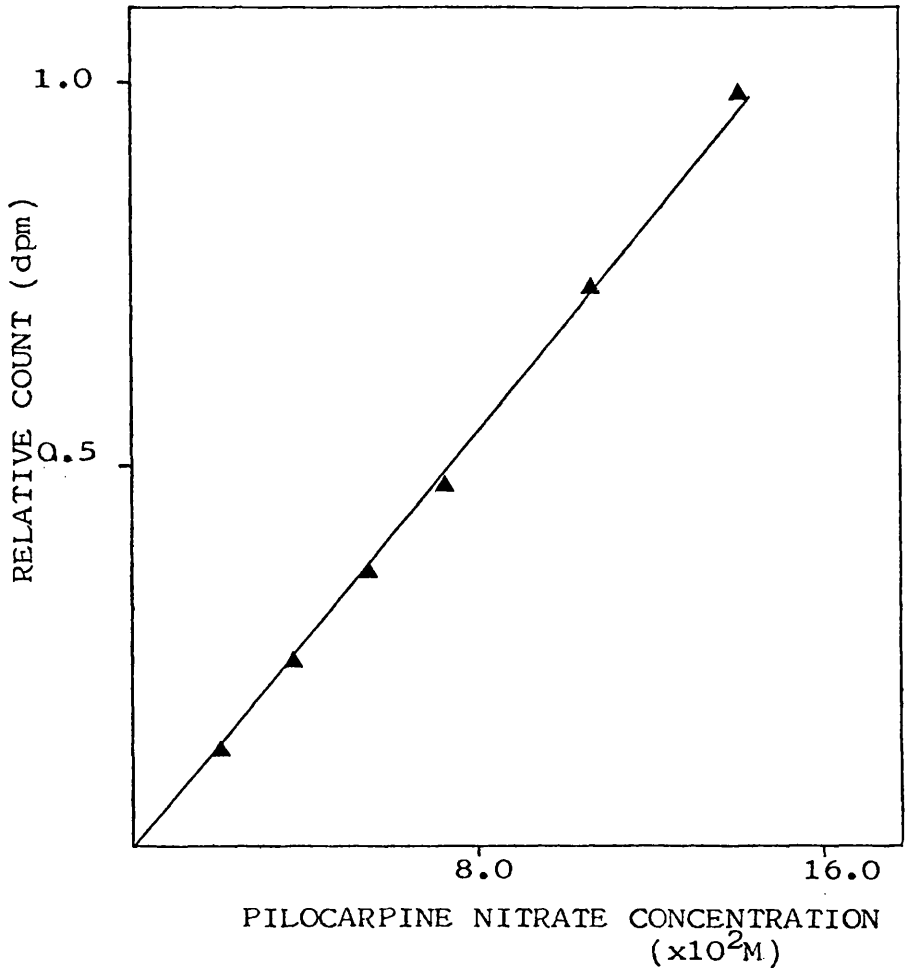


FIGURE 2.4 CALIBRATION CURVE FOR THE LIQUID SCINTILLATION COUNTING ASSAY OF PILOCARPINE NITRATE.

Slope	7.124167×10^4
Intercept	-1.276021
Correlation Coefficient	0.9990467
Standard Deviation of Slope	1.556553×10^3
Standard Deviation of Intercept	1.336769×10^2

TABLE 2.5 STATISTICAL DATA FOR THE CALIBRATION CURVE FOR THE LIQUID SCINTILLATION COUNTING ASSAY OF PILOCARPINE NITRATE.

Specific activity of tritiated pilocarpine $10.0 Ci\ mmol^{-1}$

Typical total activity of 10ml of initial stock solution 5×10^5 dpm

$1\mu Ci = 3.7 \times 10^4$ disintegrations per second $= 2.22 \times 10^{-6}$ dpm

Therefore 5×10^5 dpm $= \frac{5 \times 10^5 \times 10^{-6}}{2.22}\ \mu Ci = 0.23\mu Ci$

$0.23\mu Ci \equiv 0.23 \times 10^{-10}$ moles tritiated pilocarpine

Typical concentration of unlabelled pilocarpine nitrate in stock solution $= 1.85 \times 10^{-2}M$

Therefore total mass of unlabelled pilocarpine in 10ml = 1.85×10^{-4} moles

It may be seen from the above calculation that the mass of labelled pilocarpine added is negligible relative to the total mass of unlabelled pilocarpine nitrate present. Similar calculations for subsequent assays were therefore not carried out.

2.4.1.3 Assay of Pilocarpine Nitrate by High Pressure Liquid Chromatography

The method used for the assay of pilocarpine nitrate by HPLC was based on a method described by Noordam and others (131).

Conditions

Column	Hypersil 5 C_{18} 10cm x 0.5cm ID (approximately 5,000 plates)
Mobile Phase	Double distilled water: methanol 97:3 containing potassium dihydrogen orthophosphate 5% w/v pH adjusted to 2.5 with orthophosphoric acid
Flow rate	$2.5ml\ min^{-1}$
Wavelength for detection	215nm
Injection volume	20µl

Verification of HPLC System

To ensure separation of pilocarpine and its degradation products was achieved using the above HPLC system, 20µl of a solution containing pilocarpine nitrate, isopilocarpine and degraded solutions of the above was injected onto the column. Retention times are given in Table 2.6 and a typical chromatogram is shown in Figure 2.5. Pilocarpine and isopilocarpine were degraded by heating a 3.69×10^{-2}M solution in 0.1M sodium hydroxide at 90^{o}C for 1 hour.

Table 2.6 Retention Times for Pilocarpine Nitrate and its Degradation Products Conditions as p 90

Compound	Retention Time (minutes)
Isopilocarpine	5.93
Pilocarpine nitrate	6.73
Degraded pilocarpine nitrate (assumed to be pilocarpic acid)	7.77
Degraded isopilocarpine (assumed to be isopilocarpic acid)	8.53

From Figure 2.5 it may be seen that under the conditions stated separation of pilocarpine nitrate and its degradation products was not complete. Separation however, was not improved by altering the composition of the mobile phase such as changing the water methanol ratio, altering the amount of potassium dihydrogen orthophosphate present or adjusting the pH. Since the proportion of degradation products in the pilocarpine nitrate assayed was expected to be small it was unlikely that these would interfere with the assay values obtained despite incomplete separation of the peaks. To ensure this hypothesis was correct

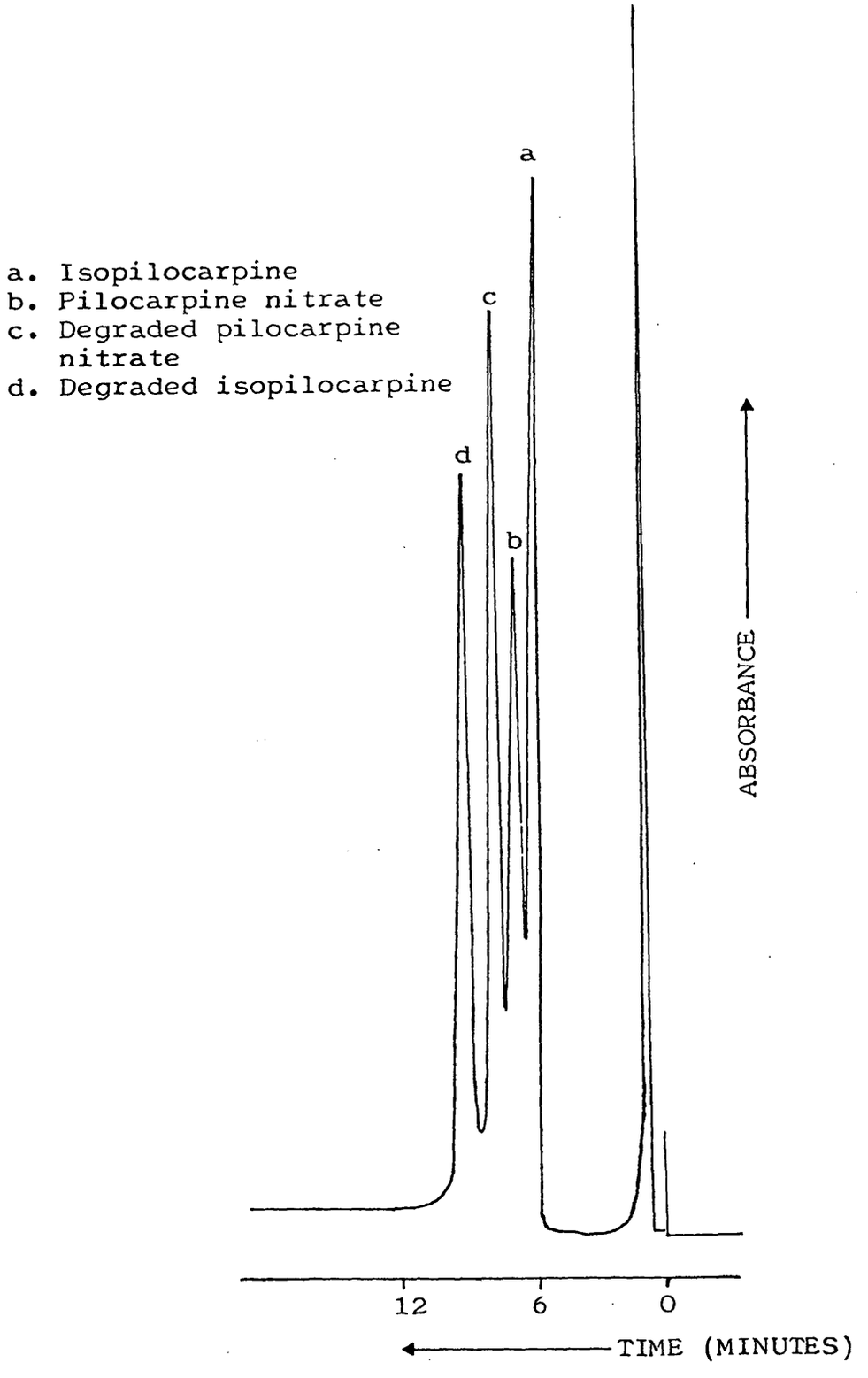


FIGURE 2.5 HPLC TRACE OF A MIXTURE OF PILOCARPINE NITRATE, ISOPILOCARPINE AND DEGRADED SAMPLES OF THE TWO COMPOUNDS.

the calibration curve for the pilocarpine nitrate assay was determined in the presence of isopilocarpine (see below).

Calibration Curve for Pilocarpine Nitrate

To determine if there was a linear reltionship between the concentration of pilocarpine nitrate and the equivalent peak heights obtained using the above HPLC system it was necessary to construct a calibration curve plot. A series of aqueous solutions of known concentrations of pilocarpine nitrate and isopilocarpine were prepared such that the total concentration of the two isomers was 9×10^{-4}M. 20µl of each solution was injected onto the column and the peak heights obtained corresponding to pilocarpine nitrate measured.

Figure 2.6 shows there is a linear relationship between pilocarpine nitrate concentration and peak height and the presence of the isopilocarpine had no apparent effect on the assay. The data were submitted to a computerised least squares regression analysis and the values of the slope and intercept together with their associated standard deviations are given in Table 2.7. Although the intercept is not zero the curve passes through the origin within one standard deviation. On the basis of these findings it was considered that measurement of concentration by determination of peak height in this case was satisfactory.

As column performance may vary with use, the slope of the calibration curve cannot always be used with confidence to determine the concentration of pilocarpine nitrate. In

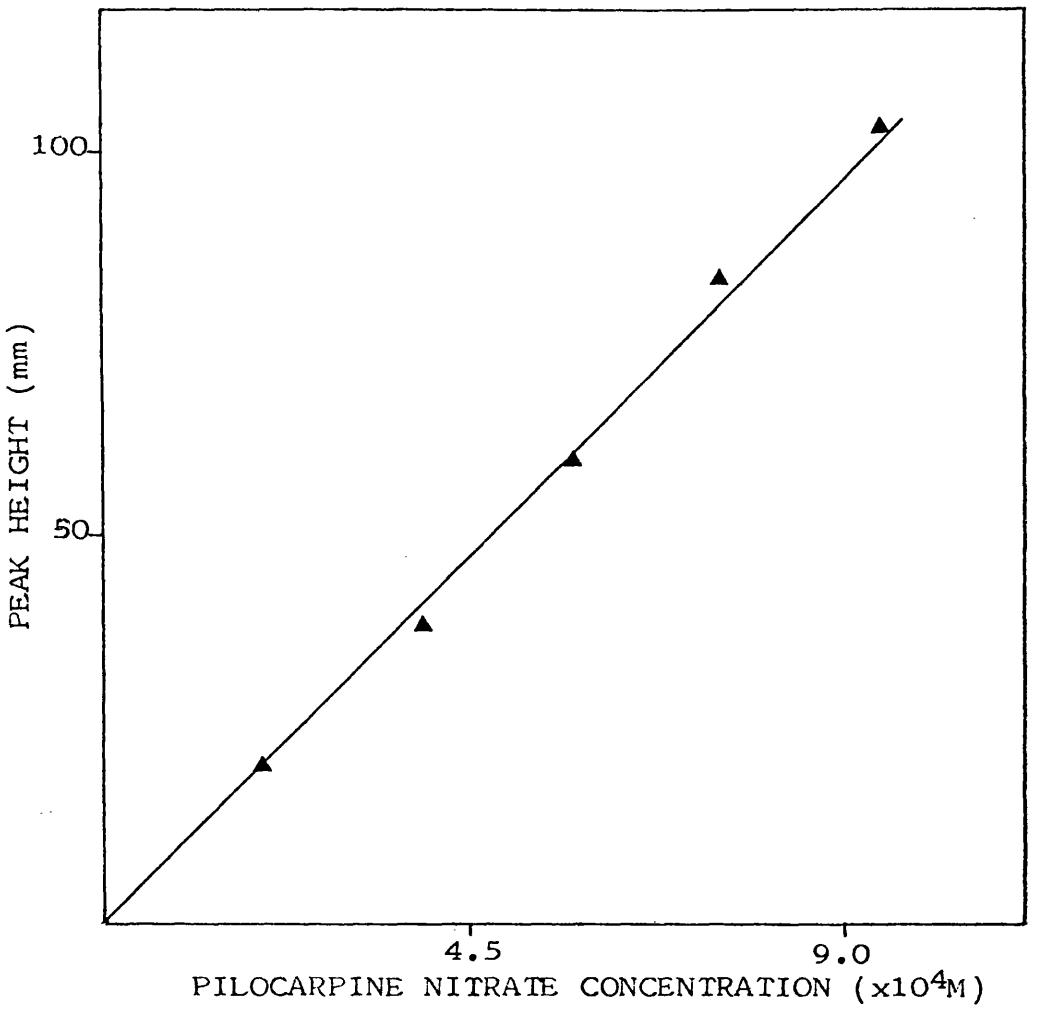


FIGURE 2.6 CALIBRATION CURVE FOR THE HPLC ASSAY OF PILOCARPINE NITRATE.

Slope	1.133137×10^5
Intercept	-0.7539099
Correlation Coefficient	0.9982942
Standard Deviation of Slope	3.826069×10^3
Standard Deviation of Intercept	2.373931

TABLE 2.7 STATISTICAL DATA FOR THE CALIBRATION CURVE FOR THE H.P.L.C. ASSAY OF PILOCARPINE NITRATE.

practice therefore, duplicate standard solutions of a known pilocarpine nitrate concentration were injected to 'bracket' injections of the test sample. In this way the concentration of pilocarpine nitrate in the test solution could be calculated with reference to the standard solution.

2.4.2 Assay of Prednisolone Sodium Phosphate by HPLC

The method used for the assay of prednisolone sodium phosphate was based on a method described by Stroud and others (133).

Conditions

Column	Partisil 10 SAX 10cm x 0.5cm ID
Mobile Phase	10% v/v methanol in 0.02 molar McIlvaines citrate - phosphate buffer pH 5.2
Flow Rate	1.5ml min^{-1}
Wavelength of detection	254 nm
Injection volume	20µl

A typical chromatogram is shown in Figure 2.7.

Calibration Curve for Prednisolone Sodium Phosphate

A calibration curve for the assay of prednisolone sodium phosphate by HPLC was determined in order to verify a linear relationship between peak height and prednisolone sodium phosphate concentration. A series of aqueous solutions of known concentrations of the drug were prepared. 20µl of each solution was injected onto the column and the heights of the peaks obtained measured. Calibration data are given in Figure 2.8 and Table 2.8 from which it may be seen that there is

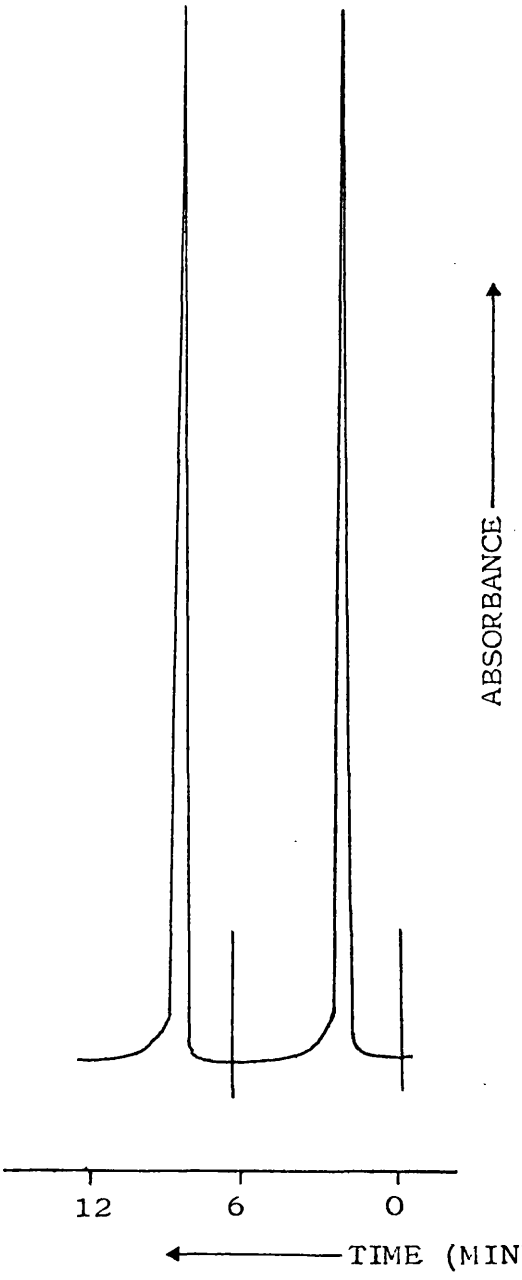


FIGURE 2.7 HPLC TRACE OF DUPLICATE INJECTIONS OF PREDNISOLONE SODIUM PHOSPHATE.

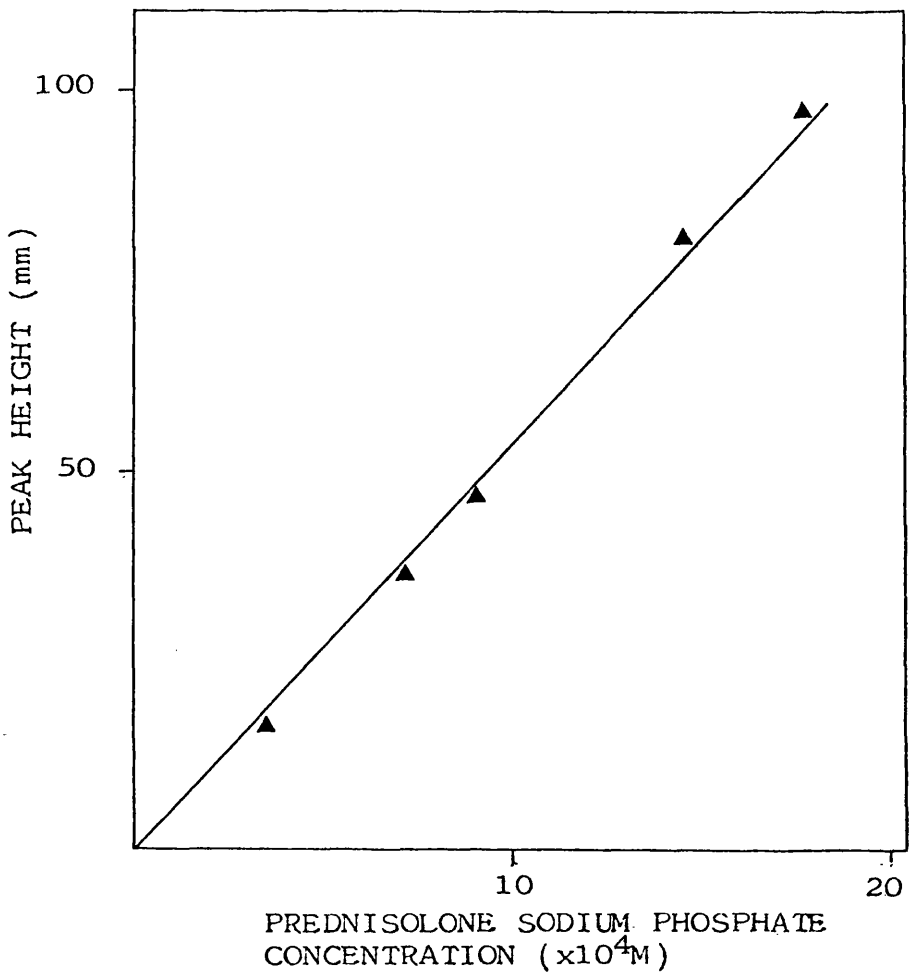


FIGURE 2.8 CALIBRATION CURVE FOR THE HPLC ASSAY OF PREDNISOLONE SODIUM PHOSPHATE.

Slope	6.085509×10^4
Intercept	-5.673298
Correlation Coefficient	0.9941774
Standard Deviation of Slope	3.2997940×10^3
Standard Deviation of Intercept	4.719850

TABLE 2.8 STATISTICAL DATA FOR THE CALIBRATION CURVE FOR THE H.P.L.C. ASSAY OF PREDNISOLONE SODIUM PHOSPHATE.

a linear relationship between peak height and prednisolone sodium phosphate concentration over the range studied. The line does not however pass through the origin although the value of the intercept is within two standard deviations of the origin. As discussed in the HPLC assay of pilocarpine nitrate, the calibration curve was not used to determine solution concentration. In practice, duplicate standard solutions of known prednisolone sodium phosphate concentration were injected to 'bracket' injections of the test samples and the test samples assayed with reference to the standard solutions.

2.4.3 Colorimetric Assay of Ethyl-4-aminobenzoate

Ethyl-4-aminobenzoate was assayed using a modified Bratton-Marshall reaction (134). To 3ml aliquots of ethyl-4-aminobenzoate solutions over the concentration range 2 to 10 x 10^{-5}M was added 3ml 0.1M HCl and 1ml 0.1% w/v sodium nitrite solution. After shaking for 5 minutes 1ml 2% w/v ammonium sulphamate solution was added and the solutions shaken for a further 2 minutes. 1ml 0.1% w/v N-1 naphthylethylenediamine solution was then added. The volume was adjusted to 25ml with distilled water and the colour allowed to develop, in the dark, for at least 15 minutes. The absorbance of each solution at the wavelength of maximum absorbance was then determined against a blank prepared as above but omitting the ethyl-4-aminobenzoate. The wavelength of maximum absorbance (λ_{max}) was reported by Bray (134) and in this study found to be 554nm.

Calibration Curve for Ethyl-4-aminobenzoate

In order to demonstrate that there was a linear relationship between ethyl-4-aminobenzoate concentration and absorbance a calibration curve was constructed. Solutions of ethyl-4-aminobenzoate of known concentrations over the range 2 to 10 x 10^{-5}M were prepared and assayed for ethyl-4-aminobenzoate. Calibration data are given in Figure 2.9 and Table 2.9. In both determinations the intercepts are within one standard deviation of the origin and the calibration curves show high linear correlation coefficients. The values of the two slopes obtained were compared using a t-test (Appendix 2). The two values are not significantly different at the 95% level of significance. The colorimetric assay was therefore considered satisfactory for the assay of ethyl-4-aminobenzoate.

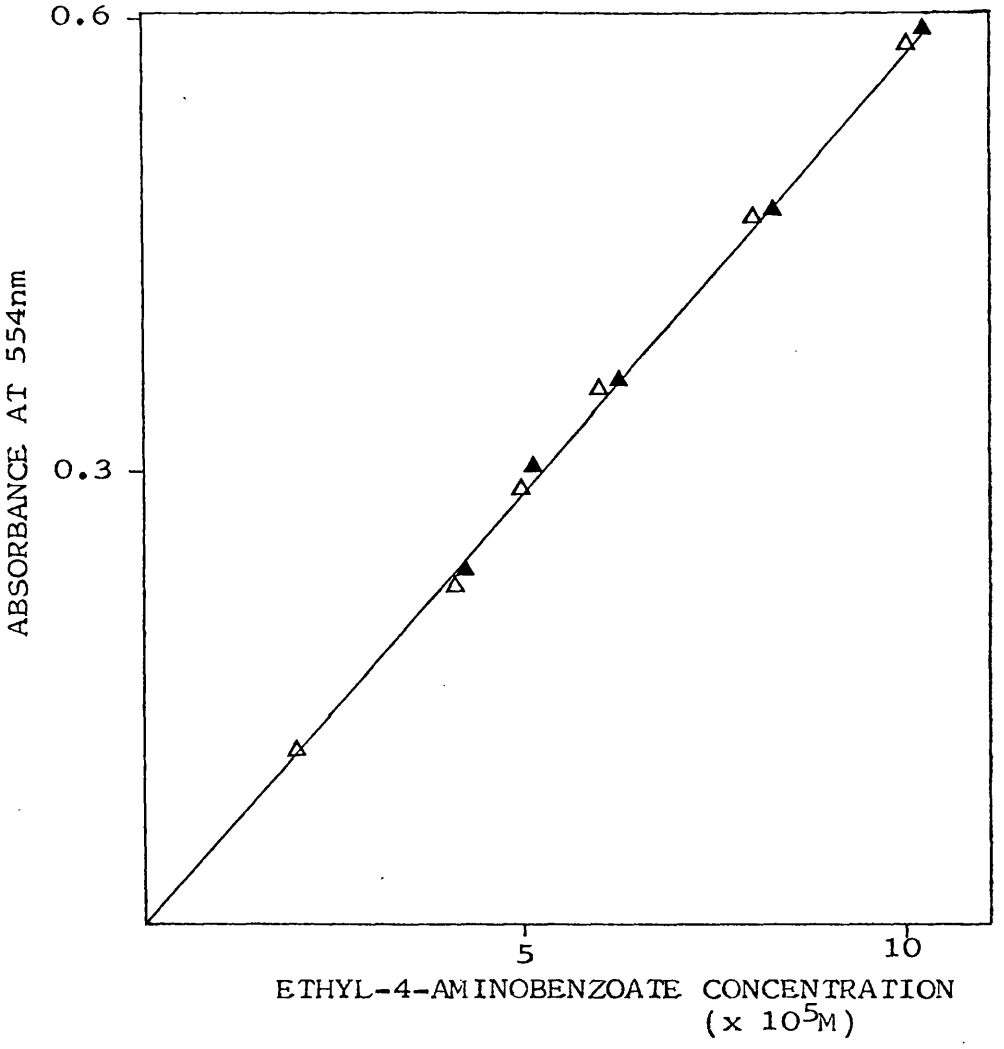


FIGURE 2.9 CALIBRATION CURVE FOR THE COLORIMETRIC ASSAY OF ETHYL-4-AMINOBENZOATE.

	Determination 1	Determination 2
Slope	5.817875×10^{3}	5.751355×10^{3}
Intercept	-5.431687×10^{-3}	-3.534903
Correlation Coefficient	0.9997886	0.9992259
Standard Deviation of Slope	5.980708×10^{1}	1.307318×10^{2}
Standard Deviation of Intercept	3.796075×10^{-2}	9.252293×10^{-3}

TABLE 2.9 STATISTICAL DATA FOR THE CALIBRATION CURVE FOR THE COLORIMETRIC ASSAY OF ETHYL-4-AMINOBENZOATE.

EXPERIMENTAL

3.1 Preparation of Nylon 6.10 Microcapsules

3.1.1 Development of Method

Initial attempts to prepare nylon 6.10 microcapsules were based on the method of Chang and others (29) which is described in Section 1.5.2.1 and is shown schematically in Figure 1.7. The compositions of the solutions were the same as those reported by Chang and others except that no haemolysate preparation was used. Solutions were cooled to 4°C by immersing in ice for approximately 1 hour prior to use. Emulsion formation, which was carried out in a 100ml glass beaker, was effected using a magnetic stirrer for the time stated in Section 1.5.2.1 and following addition of the sebacoyl chloride solution polymerisation was allowed to continue for 3 minutes. 'Microcapsules' were harvested by centrifugation, washing in Tween 20 solution and resuspended in sodium chloride solution. Microscopic examination of the final suspension in saline revealed the presence of small solid particles believed to be nylon 6.10 polymer. Also present were a large number of emulsion droplets thought to have arisen from the transfer of organic solvents to the aqueous medium during the harvesting procedure. As discussed in Section 1.5.1, there are many factors which may influence both nylon 6.10 polymerisation and microcapsule formation. These include such factors as emulsion formation and stability, composition of the organic phase, ratio of monomers present and so on. A number of modifications to the method outlined above were therefore carried out to achieve satisfactory preparation of nylon 6.10 microcapsules. These investigations are briefly described in Section 3.1.1.1. Throughout the development procedure sebacoyl chloride solutions

were prepared immediately prior to use and the entire volume added rapidly to the formed emulsion.

3.1.1.1 Modifications to Initial Method

A. Method of Harvesting: Microcapsules prepared by interfacial polymerisation techniques are thin walled (27) and it was thought possible that centrifugation may have caused the formed microcapsules to disintegrate. Also, the 50% Tween 20 solution used by Chang and others to disperse the microcapsules in an aqueous medium may, even on dilution, have been too viscous to achieve satisfactory separation of the microcapsules by centrifugation. Using the method of preparation described in Section 3.1.1 the following methods of harvesting were investigated:

i) after quenching, the contents of the beaker were passed through phase separating paper.

ii) a 50% v/v solution of a non-ionic surfactant. Pluronic L64 was used instead of Tween 20 solution.

iii) the 'microcapsule' suspension was centrifuged, washed with ethanol or acetone, centrifuged again and then transferred to 0.9% w/v sodium chloride solution.

iv) procedure (iii) above was used except that after washing in ethanol or acetone the suspension was dispersed in 50% v/v Tween 20 solution which was then diluted with water, centrifuged and the 'microcapsules' transferred to 0.9% sodium chloride solution.

v) cyclohexane was used to quench the reaction and the microcapsules harvested by either the initial method, method (iii) or method (iv).

vi) the contents of the beaker were rotary evaporated for 1 hour at 30°C to form a slurry which was then dispersed in ethanol, centrifuged and redispersed in sodium chloride solution.

In all cases no microcapsules were observed. The suspensions obtained consisted of small, solid particles together with emulsion droplets. Method (iv), however, resulted in little transfer of organic solvents into the final aqueous dispersion medium and was therefore adopted as the method of harvesting for further development work.

B. Emulsion Formation: One of the factors which varied between different reported methods of polyamide microcapsule preparation was the method used to form the emulsion (Section 1.5.2.2). The procedure outlined in Section 3.1.1, except for the modified harvesting method, was therefore investigated using different methods of emulsion formation. Samples were removed for microscopical examination at two stages (a) from the organic phase prior to the addition of the sebacoyl chloride and (b) from the final suspension in saline. The methods of forming the emulsion studied were:

i) an ultrasonic probe (Rapidis 300 Ultrasonics Ltd.) was used to form the emulsion which was then transferred to the glass beaker.

ii) A Silverson stirrer fitted with an homogeniser attachment (Silverson Machines Ltd.) was used at various speed settings to prepare the emulsion before transferring to the glass beaker.

iii) a Silverson stirrer fitted with an homogeniser attachment was used throughout the preparation.

iv) an overhead Citenco stirrer motor fitted with a three bladed plastic paddle at various speed settings over the range 200 to 2,000 rpm was used throughout the preparation.

In all cases, samples removed at stage (a) were found to be stable emulsions with droplet size of less than 200μm. However, again no microcapsules were formed. The use of a glass beaker was found to be inconvenient for many of these methods of emulsion formation. Therefore, for subsequent investigations a 100ml capacity round-bottom flask was used together with a Citenco overhead stirrer fitted with a paddle.

C. Composition of Reactant Solutions: In the original method Chang and others (29) selected an organic phase of chloroform: cyclohexane 1:4 as its density was approximately the same as that reported for the microcapsules. This was believed to minimise damage and allow separation of the microcapsule by centrifugation. The composition of the reactant solutions however has been altered by a number of workers (Section 1.5.2.2) and has still resulted in the formation of microcapsules. Various combinations of surfactant, monomer concentrations and organic phase compositions were therefore investigated over the range outlined in Table 3.1. None of these resulted in the formation of microcapsules however.

D. Reaction Conditions: Other factors which may influence polymerisation and therefore microcapsule formation are the temperature of the reaction system, the internal phase volume of the emulsion and the duration of the reaction prior to quenching. The effect of these reaction conditions on microcapsule formation was examined

Table 3.1 Parameters varied in the Investigation of the Effect of Composition of Reactant Solutions on Nylon 6.10 Microcapsule Formation

Variable	Range			
Chloroform:cyclohexane ratio by volume	1:3		1:4	1:5
Surfactant concentration: Span 85	0	1	5	10% v/v
Surfactant concentration: Arlacel A		1		10% v/v
Sebacoyl Chloride concentration	0.009		0.018	0.037M
1,6 hexamethylenediamine concentration	0.2		0.4	0.8M

using different combinations of organic phase solvent ratio, surfactant concentration and monomer concentration as outlined in Table 3.1. For each reaction time of 1, 3 and 10 minutes the experiment was carried out surrounded by ice and at room temperature. The emulsification time was also varied between 30 seconds and 3 minutes. In addition the relative phase volumes were changed as described in Table 3.2. No microcapsules were observed to be formed in any of the above cases.

E. Presence of Additives: As discussed in Section 1.5.2.2 several workers have included additives in the internal aqueous phase of the microcapsules such as polyethylene glycol 400 (PEG 400) (93), polyethyleneimine (94) and bovine serum albumin (105).

Table 3.2 Phase Volume Ratios Investigated in the Preparation of Nylon 6.10 Microcapsules

	1	2	3
Volume of internal aqueous phase	5ml	1.5ml	2.5ml
Volume of organic phase prior to addition of sebacoyl chloride solution	25ml	7.5ml	25ml
Volume of sebacoyl chloride solution	25ml	7.5ml	25ml

The procedures used previously for preparing microcapsules were therefore investigated with the inclusion of these additives. The conditions used and the amount of additives present are described in Table 3.3. The reaction was quenched by pouring the suspension produced after the addition of the sebacoyl chloride solution into chloroform: cyclohexane 1:3 and harvesting was achieved by washing in acetone followed by Tween 20 solution. 'Microcapsules' formed were resuspended in saline.

In the presence of 10% w/v polyethyleneimine, structures resembling microcapsules were observed in the final aqueous medium. Their size was approximately 200μm. However,

the microcapsules were not spherical but had the appearance of collapsed hollow 'sacks'.

Table 3.3 Summary of Conditions used to Investigate the Method of Preparation of Nylon 6.10 Microcapsules in the Presence of Various Additives

Additive Concentration PEG 400 Polyethyleneimine Bovine Serum Albumin	 0.5% v/v 10% w/v 0.1% w/v
Aqueous Phase Composition	5ml of 0.2M solution of 1,6 hexamethylenediamine in carbonate-bicarbonate buffer pH9.8.
Organic Phase Composition	25ml of chloroform: cyclohexane 1:3 by volume containing 10% v/v Span 85
Sebacoyl Chloride Solution Composition	25ml of 0.019M sebacoyl chloride in above organic phase
Stirring speed	approximately 500rpm
Emulsification Time	3 minutes
Reaction Time	3 minutes
Temperature	approximately 4°C

Capsular structures were not observed in the presence of bovine serum albumin or PEG 400. It was therefore apparent that satisfactory microcapsules might be prepared in the presence of a crosslinking agent, polyethyleneimine, and the use of this crosslinking agent was further investigated.

3.1.1.2 Modifications to the Preparation of Microcapsules using Polyethyleneimine, a Crosslinking Agent

It has been reported that particles of less than approximately 20μm cannot be felt when instilled in the eye (135). Therefore, in order to produce a suspension of microcapsules suitable for

ophthalmic administration it was necessary to develop a method for producing smaller microcapsules. This was based on the method developed in Section 3.1.1.1. Microcapsule size was assessed using a light microscope fitted with a calibrated eye piece micrometer. Using the conditions set out in Table 3.3 for the successful preparation of polyethyleneimine containing microcapsules as a basis for further modifications, the parameters shown in Table 3.4 were investigated. Initially the method of emulsion formation was examined. Microcapsules were not formed if a magnetic stirrer, Silverson stirrer with homogeniser attachment or ultrasonic probe were used instead of the overhead stirrer. Increasing the speed of stirring of the overhead stirrer and changing the reaction vessel from a round bottom flask to a cylindrical centrifuge tube both decreased the microcapsule size and improved their appearance. Using the overhead stirrer in combination with the centrifuge tube and stirring at 2,000 rpm the compositions of the aqueous and organic phases were then examined. Various combinations of monomer concentration, polyethyleneimine concentration, Span 85 concentration and so on were investigated as described in Table 3.4. Finally, using several of these combinations the reaction conditions and method of harvesting were examined (Table 3.4). Well formed microcapsules of 20 to 30μm diameter were produced using 5% w/v polyethyleneimine solution, 0.019M sebacoyl chloride, 0.4M 1,6 hexamethylenediamine solution and a chloroform: cyclohexane ratio of 1:3 by volume containing 1% v/v Span 85. The emulsion was formed using an overhead motor with paddle stirrer rotating at 2,000 rpm and the reaction vessel was a 100ml capacity centrifuge tube surrounded by ice.

Table 3.4 Modifications to the Preparation of Nylon 6.10 Microcapsules Prepared using Polyethyleneimine

Variable	Range
Emulsification Conditions	
Mechanical emulsifier	- ultrasonic probe homogeniser magnetic stirrer overhead stirrer
Speed of overhead stirrer	- 500 - 2,000 rpm
Reaction vessel used in combination with overhead stirrer	- round bottom flask - 100ml centrifuge tube
Aqueous and Organic Phase Composition	
Polyethyleneimine concentration	1, 5, 10% w/v
1,6 hexamethylenediamine concentration	0.2, 0.4, 0.8M
Sebacoyl chloride concentration	0.009, 0.019, 0.03M
Chloroform: cyclohexane ratio	1:3 1:4 1:5
Span 85 concentration	1.0 5.0 10.0% v/v
Reaction and harvesting Conditions	
Reaction temperature	Room temperature $4^{o}C$
Emulsification time	1, 3, 5 minutes
Reaction time	1, 3, 5, 10 minutes
Quenching solution	Chloroform: cylochexane 1:3, 1:4 or 1:5 or 100% cyclohexane
Harvesting method	Centrifugation from organic phase and dispersion in Tween 20 solution or, wash in acetone before dispersion in Tween 20 solution.

The emulsification and reaction times were 1 minute and 3 minutes respectively and cyclohexane was used to quench the reaction. Harvesting was facilitated if acetone was used to transfer the microcapsules to the aqueous Tween 20 solution. No microcapsules were formed if the polyethyleneimine was omitted but above 1% w/v the polyethyleneimine concentration had no detectable effect on microcapsule appearance. Altering the concentration of the monomers and the chloroform: cyclohexane ratio also had

no obvious effect on microcapsule appearance. Furthermore, no effect on appearance was observed on altering the reaction temperature or reaction and emulsification times. Recovery of the microcapsules from the organic phase was aided if the quenching solution was cyclohexane alone and the microcapsules showed no differences from those quenched using the appropriate external organic phase. Increasing the Span 85 concentration from 1% to 10% had no significant effect on microcapsule size whilst stirring at 2,000 rpm.

Attempts to further reduce the size of the microcapsules by increasing the stirring speed above 2,000 rpm were unsuccessful as this resulted in aggregation of the microcapsules. In order to prevent or minimise this cohesion the following conditions were altered.

1. The relative volumes of the internal and external phases were altered as shown in Table 3.5.
2. The Span 85 concentration was increased from 1% v/v to 15% v/v to increase the stability of the emulsion and decrease coalescence.
3. Benzidine disulphonic acid 6.5% w/v was included in the preparation by dissolution in the aqueous phase. This is believed to give rise to negative charges on the surface of the microcapsules which aid dispersion (101).
4. The reaction time was decreased from 3 minutes to 2 minutes and 30 seconds respectively to prevent nylon forming between the microcapsules.

5. 0.5ml aniline was added to the quenching solution to rapidly stop polymerisation.
6. The volume of sebacoyl chloride added was reduced to give a concentration of 0.005M.
7. The chloroform:cyclohexane ratio was decreased to 1:19 to reduce the solubility of the diamine in the external organic phase.
8. The concentration of 1,6 hexamethylenediamine was decreased to 0.1M to reduce the total amount of nylon formed.

All attempts however to prevent aggregation of the smaller microcapsules were unsuccessful.

Table 3.5 Volumes of Internal and External Phases Investigated in the Preparation of Nylon 6.10 Microcapsules containing Polyethyleneimine

	1	2	3	4
Volume of internal phase	5ml	3ml	2.5ml	1.5ml
Volume of external phase prior to addition of sebacoyl chloride solution	25ml	15ml	25ml	30ml
Volume of sebacoyl chloride solution	25ml	30ml	25ml	30ml

'Scale-Up' of Microcapsule Preparation

Further processes developed for microcapsule preparation were based on the method described in Section 3.1.1.2. A major modification to this method was the increase in weight and volume of reactants used. This was carried out in order to increase the yield of microcapsules obtained. To accommodate the increased volume of reactant solutions it was necessary to further change the reaction vessel from a 100ml capacity

centrifuge tube to a 250ml conical flask. It was observed throughout the development procedure that microcapsule appearance improved for a given system if the sebacoyl chloride had been recently redistilled. For this reason the diacid chloride was redistilled approximately every four weeks and stored under nitrogen. It was also found that microcapsule suspensions could successfully be stored in water in place of saline.

As a result of the investigations described above it was apparent that to produce microcapsules with diameters of approximately 20-30μm several factors had to be taken into account. These included the nature of the mechanical stirrer, the type of reaction vessel, the use of a crosslinking agent, polyethyleneimine and the stirring speed. The method finally selected for microcapsule preparation is described in detail in Section 3.1.2.

3.1.2 Method of Preparation of Nylon 6.10 Microcapsules

The following solutions were prepared and cooled to 4°C before use:

Solution 1 Equivolume 0.9M carbonate-bicarbonate buffer pH 9.8 and 20% w/v aqueous polyethyleneimine solution

Solution 2 0.4M 1,6 hexamethylenediamine in distilled water

Solution 3 Chloroform:cyclohexane 1 part to 5 parts by volume containing 1% v/v Span 85

Solution 4 0.015M sebacoyl chloride in chloroform:cyclohexane 1 part to 5 parts by volume containing 1% v/v Span 85

3.75ml 'Solution 1' and 3.75ml 'Solution 2' were pipetted into a 250ml conical flask surrounded by ice and stirred at 2,000 rpm using an overhead paddle stirrer connected to a Citenco motor. Whilst stirring, 75ml 'Solution 3' was added and stirred for 1

minute after which time 75ml 'Solution 4' (prepared 20 seconds before use) was added and stirred for 3 minutes. The suspension was then poured into 100ml cyclohexane, centrifuged at 2,500 rpm for 30 seconds and the supernatant removed. The microcapsules were washed using 500ml cylcohexane, centrifuged as above and washed with 50ml acetone. After centrifuging again the slurry was dispersed in 15ml 50% aqueous Tween 20 solution and stirred for 30 seconds. This was then diluted to 150ml with distilled water. Microcapsules were removed from the Tween 20 solution by centrifugation again at 2,500 rpm and were resuspended in water. Alternatively the microcapsules were removed from the cyclohexane wash by centrifugation, washed in 50ml acetone and resuspended in water.

3.2 Preparation of Polyphthalamide Microcapsules

The method for the preparation of polyphthalamide microcapsules was based on that developed for nylon 6.10 microcapsules except that the monomers used were piperazine and phthaloyl chloride. It was observed, however, that by increasing the concentration of diacid chloride used from 0.015M to 0.044M the yield of microcapsules was considerably improved (see Section 3.3.3). This was not the case with the nylon 6.10 microcapsules. Increasing the sebacoyl chloride concentration to 0.044M resulted only in the formation of microcapsules surrounded by what appeared to be loosely attached low molecular weight polymer. The method used for the preparation of the increased yield of polyphthalamide microcapsules is given below.

The following solutions were prepared and cooled to $4^{o}C$ before use. Solutions 1 and 3 were as given for nylon 6.10 microcapsule preparation.

Solution 2	0.4M piperazine in distilled water.
Solution 4	0.044M phthaloyl chloride in chloroform: cyclohexane 1 part to 5 parts by volume containing 1% v/v Span 85.

The method of preparation was as for nylon 6.10 microcapsules described in Section 3.1.2. The method of transference to an aqueous phase was also the same.

3.3 Characteristics of Nylon 6.10 and Polyphthalamide Microcapsules

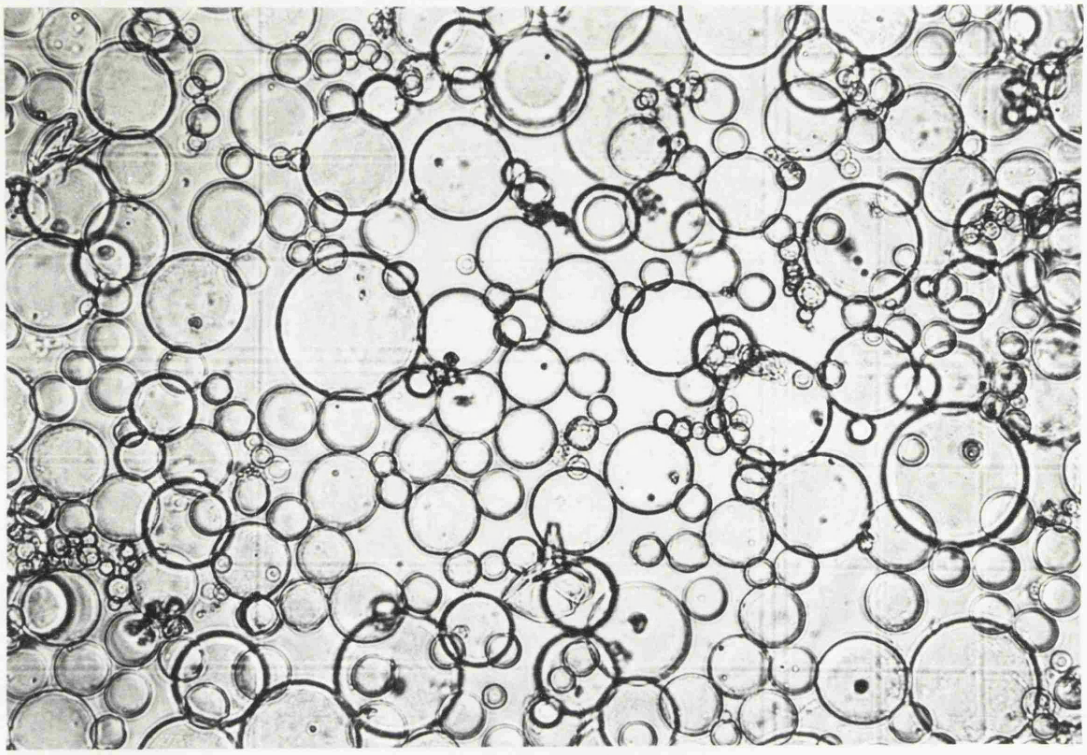
Samples of both nylon 6.10 and polyphthalamide microcapsules were prepared using the methods given in Sections 3.1.2 and 3.2 respectively and assessed for their appearance, size distribution, yield and the distribution of pilocarpine nitrate during preparation.

3.3.1 Appearance

The appearance of microcapsules was investigated using an Amplival Carl Zeiss microscope with camera attachment. Aqueous microcapsule suspensions were appropriately diluted with water and mounted in a haemocytometer: Figure 3.1 shows the appearance of nylon 6.10 microcapsules. The microcapsules are spherical with clear interiors and have thin walls relative to their diameter. A large range of sizes is present and some capsules have collapsed which is possibly due to crenation. In contrast, the polyphthalamide microcapsules shown in Figure 3.2 are more irregular in shape and have what appear to be small particles of polymer associated with them. Again, however, the microcapsules are seen to have thin walls relative to their diameter and a large size range is present.

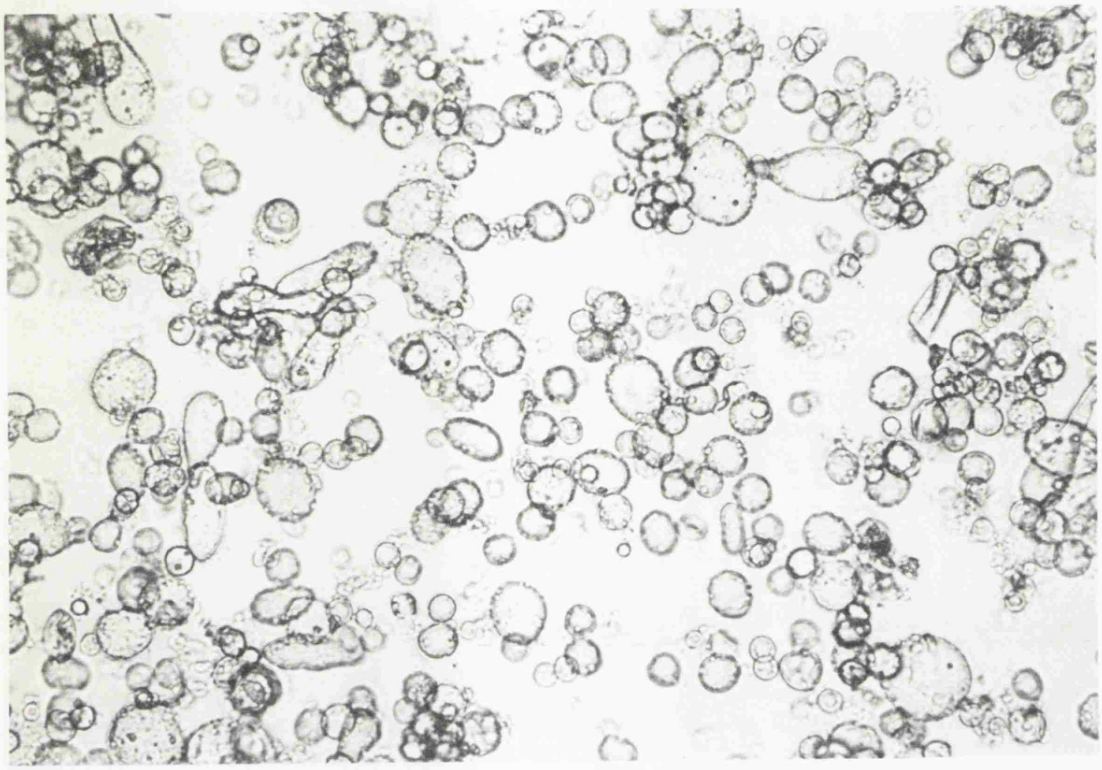
Scanning electron micrographs of these microcapsules were taken using a JEOL 35C scanning electron microscope. Microcapsules were prepared for electron microscopy by two methods.

1. Freeze Fracture: Aqueous microcapsule suspensions were rapidly frozen in Freon 22 which had previously been cooled by liquid nitrogen. The microcapsules were then transferred into liquid nitrogen. After removal,



Scale: ⊢——————⊣
40μm

FIGURE 3.1 NYLON 6.10 MICROCAPSULES DISPERSED IN WATER.



Scale: ⊢——⊣
50μm

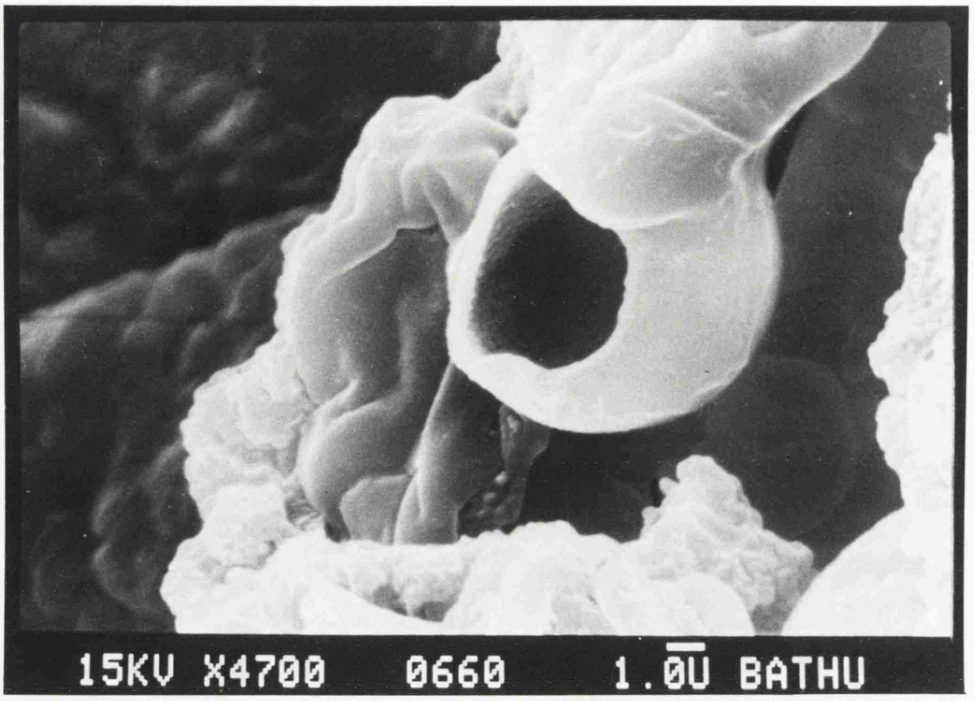
FIGURE 3.2 POLYPHTHALAMIDE MICROCAPSULES DISPERSED IN WATER.

the frozen microcapsules were fractured by placing them between two sheets of velim and striking with a hammer. Following freeze drying for 12 hours the samples were mounted and gold coated using an argon sputter coater.

2. Critical Point Drying: The sample, suspended in acetone was placed in a critical point drying chamber and the acetone replaced by liquid carbon dioxide. The chamber was then pressurised to above 1200 psi and after removal of the acetone heated to 40°C. After cooling, the microcapsules were mounted onto stubs and gold coated as above.

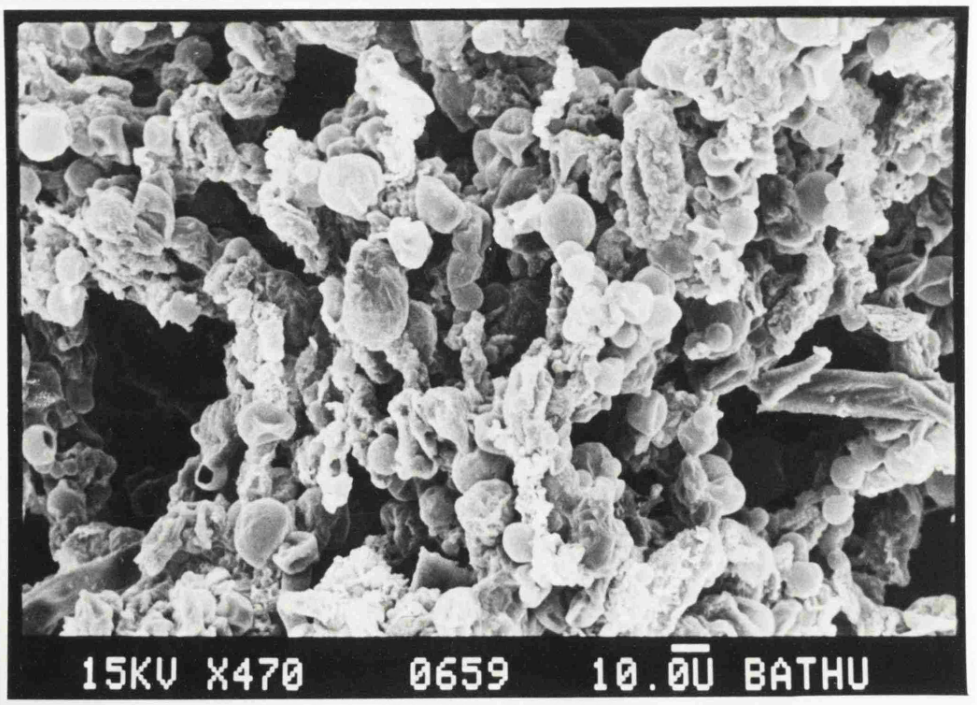
Figures 3.3a and 3.3b show respectively the appearance of a freeze fractured nylon 6.10 microcapsule and a general field of view of nylon 6.10 microcapsules from which the above micrograph was taken. The individual microcapsule is seen to be hollow and to have thin walls. The thickness of the microcapsule wall is estimated to be less than 0.5μm. The total field of view (Figure 3.3b) shows two microcapsules which have been successfully fractured, the remainder appear to be intact. From the micrograph it appears that the smaller microcapsules in the sample have remained spherical whilst the larger ones have collapsed. Figure 3.4a shows the appearance of a freeze fractured polyphthalamide microcapsule. It is again seen to be hollow and to have thin walls but it is of irregular shape. The field of view shown in Figure 3.4b is of polyphthalamide microcapsules prepared by the critical point drying technique. These microcapsules are all well reconstituted. The difference

a



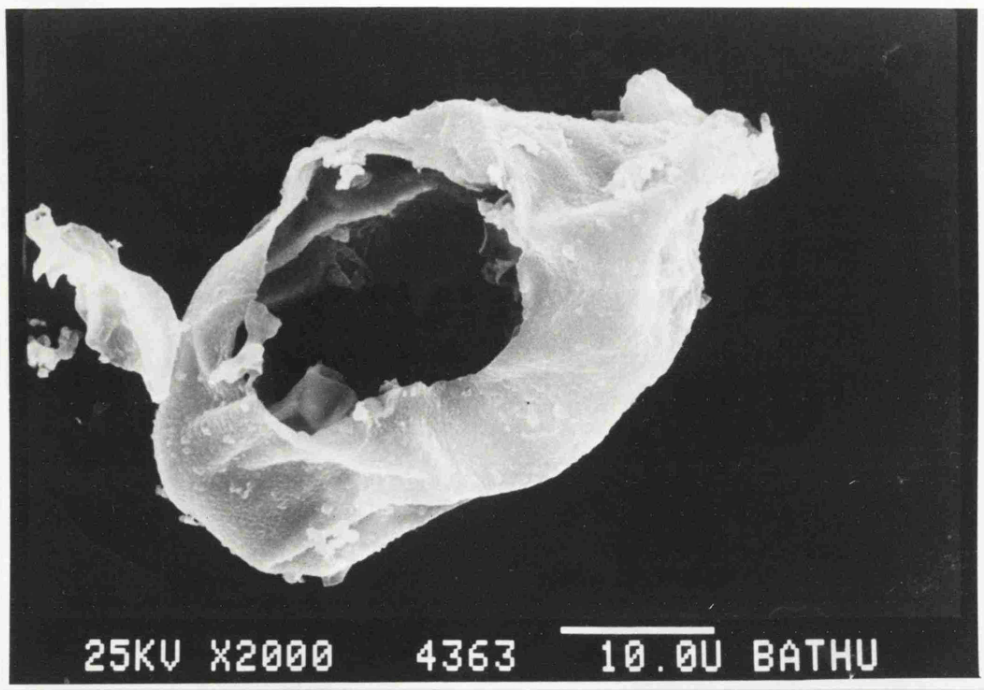
Scale: ⊢——⊣
5.0μm

b

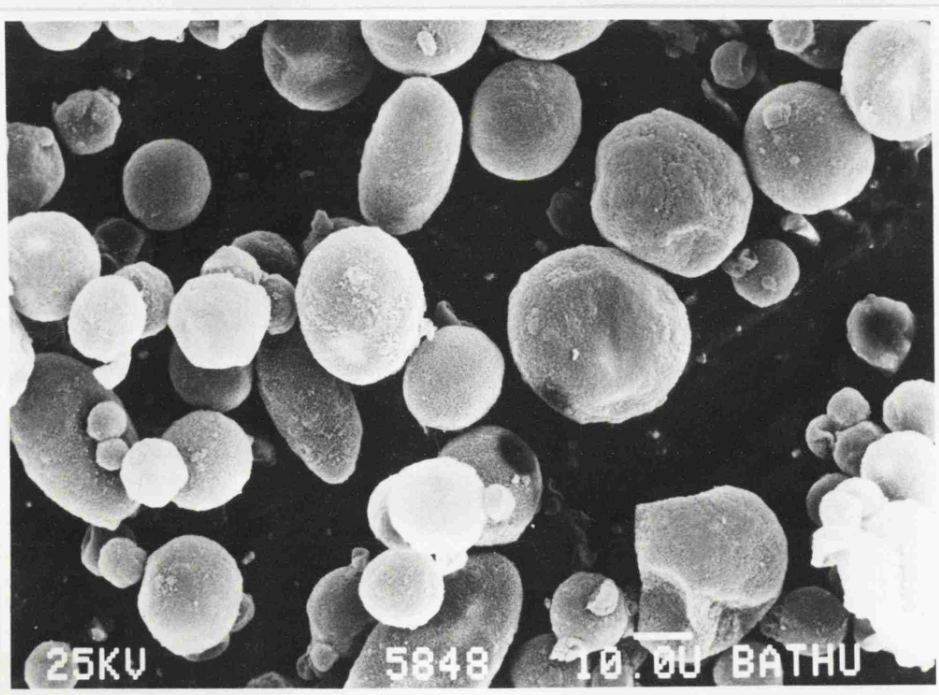


Scale: ⊢——⊣
50.0μm

FIGURE 3.3 SCANNING ELECTRON MICROGRAPHS OF FREEZE FRACTURED NYLON 6.10 MICROCAPSULES.



Scale: 10.0μm



Scale: 20.0μm

FIGURE 3.4 SCANNING ELECTRON MICROGRAPHS OF POLYPHTHALAMIDE MICROCAPSULES.

a Prepared by freeze fracture method
b Prepared by critical point drying method

between these and the collapsed microcapsules shown in Figure 3.3b may be due to the different polymeric wall material used but it is believed to be due to the preparative technique as freeze fractured polyphthalamide microcapsules have also been found to be collapsed. For this reason the critical point drying method was used for all subsequent samples to illustrate the structure of whole microcapsules. A number of the microcapsules in Figure 3.3b may again be seen to be of irregular shape.

3.3.2 Size Distribution

Size analysis was carried out using a Coulter Counter model Zb fitted with a Universal Coincidence Corrector. A 140μm orifice tube and 0.5ml volume samples were used throughout the counting. Matching switch and gain control settings were 20K and 5 respectively. Microcapsule suspensions were diluted to below 14,580 particles per 0.5ml using Isoton (Coulter Electronics Ltd., Luton) and counting carried out whilst stirring. All counts were taken in quadruplicate. Prior to its use background counts were also performed on the electrolyte used for the dilution. Solutions with total particle counts above approximately 100 per ml over the range 5 to 60μm were discarded. Calibration of the Coulter Counter was undertaken using a monodisperse suspension of pollen (Coulter Electronics Ltd.) with a nominal particle diameter of 13.53μm using the 'Half-Count Technique' (136).

In order to assess the suitability of using this method to determine the size distribution of microcapsules, the number size distribution of a given batch of polyphthalamide

microcapsules was determined using the Coulter Counter and the Fleming Particle Size Analyser. The latter is a microscopical method of size determination in which the images are sheared such that in a given direction the new image and the original image are just touching. The distance of the shear is therefore the diameter of the particle in the given direction and this distance is monitored and recorded for each particle. The frequency size distribution of polyphthalamide microcapsules determined by the two methods is shown in Figure 3.5. Comparison of (a) and (b) reveal differences in the size distribution. For example a large number of small particles 6-7.5μm were detected by the Coulter Counter but not by the Fleming Size Analyser. These differences may arise due to the diffuse images of the microcapsules obtained using the size analyser making accurate measurement of particle diameter difficult. They may also arise due to the basis of the measurement technique; that is the Coulter Counter assumes the particles are spherical in calculating the diameter of each particle. This is not the case (Figure 3.2). The cumulative frequency percent oversize curves calculated from the number size distributions are shown in Figure 3.6. It may be seen that the shapes of the curves are similar. However, the median frequency diameters were estimated to be approximately 15-16μm determined by Coulter Counter and 20μm determined by Fleming Size Analyser. The relatively small value obtained using the Coulter Counter is probably due to the significantly large number of small particles present in the sample measured. This results in a 'skew' of the distribution toward the lower values. However,

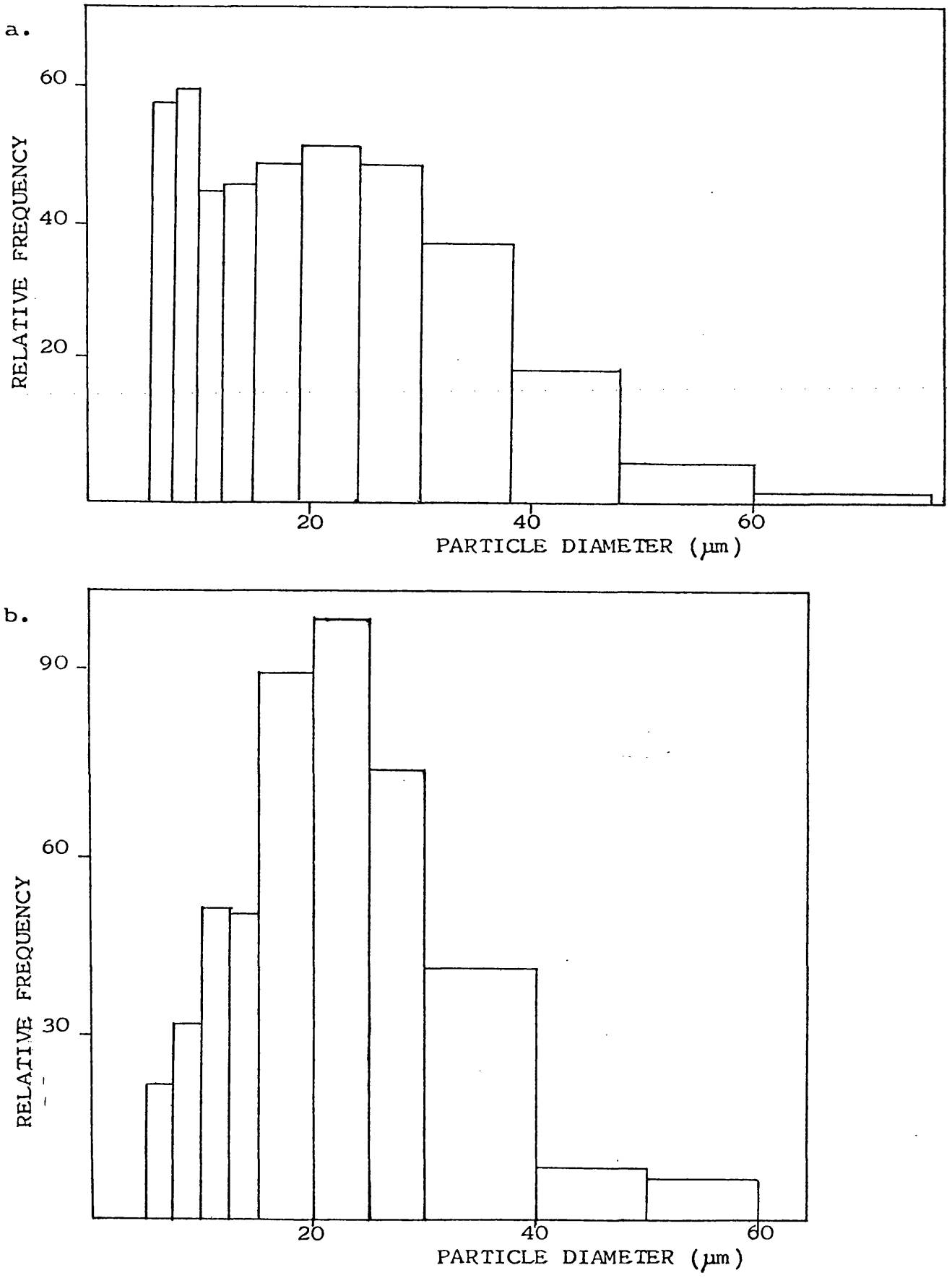
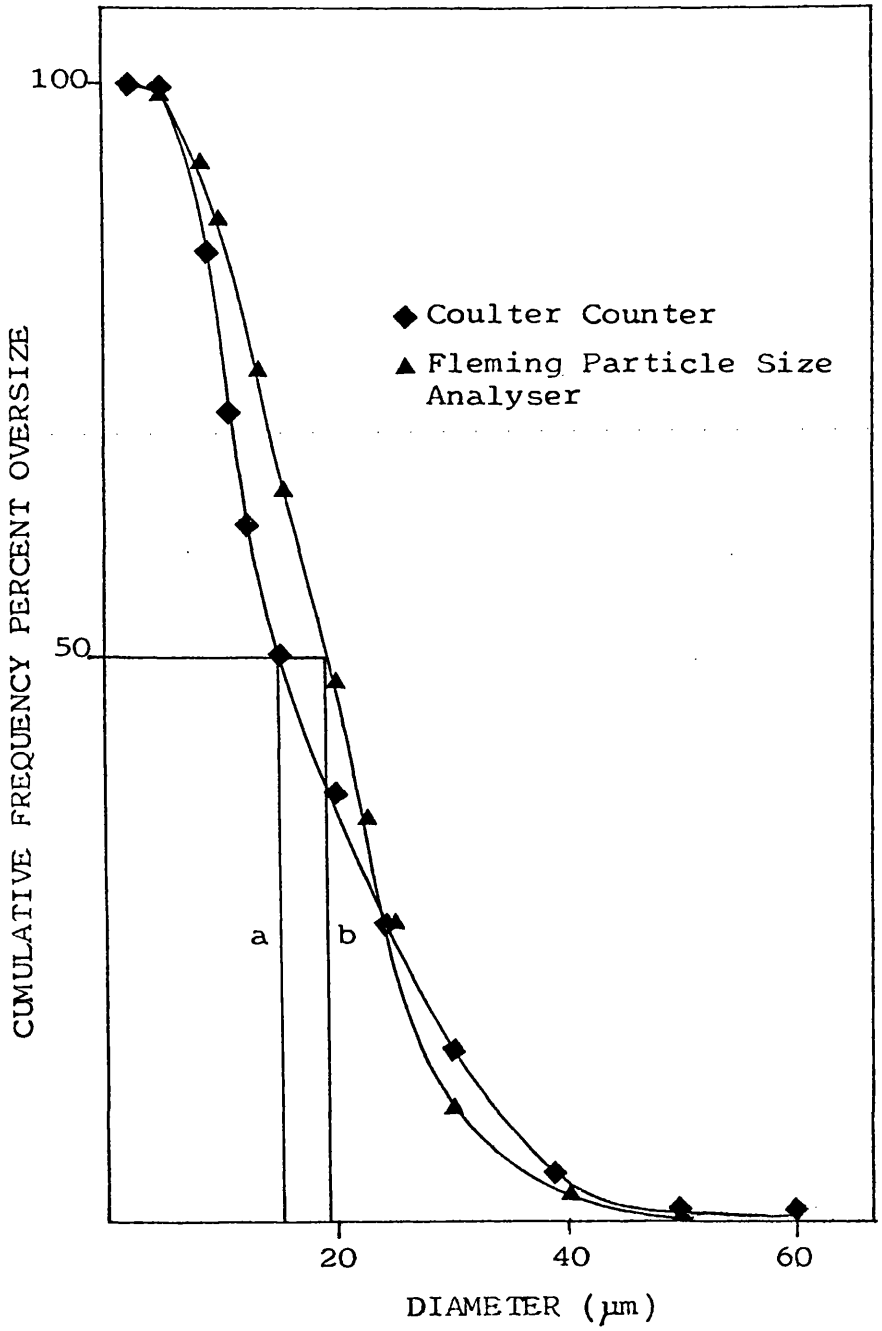


FIGURE 3.5 FREQUENCY SIZE DISTRIBUTION OF POLYPHTHALAMIDE MICROCAPSULES DETERMINED BY a) COULTER COUNTER AND b) FLEMING PARTICLE SIZE ANALYSER.



a. Median diameter determined by Coulter Counter 15-16μm.

b. Median diameter determined by Fleming Particle Size Analyser 19-20μm.

FIGURE 3.6 SIZE DISTRIBUTION OF POLYPHTHALAMIDE MICROCAPSULES DETERMINED BY COULTER COUNTER AND FLEMING PARTICLE SIZE ANALYSER.

in both cases the microcapsules are in the same size range with similar size distributions. As a result of this it was decided to use the Coulter Counter in subsequent determinations of size distribution due to its ease of operation.

Four batches of both nylon 6.10 and polyphthalamide microcapsules were prepared and their size distributions determined using the Coulter Counter. The data are given in Table 3.6 and are shown plotted as cumulative volume percent oversize curves in Figure 3.7. The diameters of the microcapsules range between approximately 5μm and 60μm and the median volume diameters for nylon 6.10 and polyphthalamide microcapsules taken from four batches are 16.5μm and 26.0μm respectively. The data obtained from all four batches are in close agreement for both types of microcapsule.

3.3.3 Microcapsule Yield

Microcapsule yield was assessed by determining the concentration of microcapsules present in a given weight of slurry obtained after rotary evaporation; where concentration is defined as the volume of microcapsules present in a given weight of slurry or suspension. For example 50% v/w refers to a total capsular volume of 0.5ml present in 1g of slurry.

Following centrifugation in cyclohexane, microcapsules were washed in 50ml acetone, centrifuged, rotary evaporated for 30 minutes at 30^{o}C and the mass of slurry obtained weighed. A known weight of this slurry was then dispersed in 200ml

Microcapsule Diameter (μm)	Cumulative Volume Percent Oversize							
	Nylon 6.10				Polyphthalamide			
	1	2	3	4	1	2	3	4
59.98	1.8	1.8	0.5	0.6	1.2	1.1	1.6	5.2
47.61	3.6	4.6	1.3	2.0	6.0	4.1	5.3	11.6
37.38	5.2	7.8	2.3	3.5	16.1	12.5	14.1	24.5
29.99	10.9	9.7	3.7	6.3	40.0	31.1	28.8	44.1
23.80	25.9	13.0	7.1	11.9	64.5	61.7	48.6	65.3
18.89	42.0	22.2	28.1	36.8	81 9	81.6	70.6	81.6
14.99	62.5	44.2	77.8	87.3	90.7	91.4	85.8	91.0
11.90	88.0	78.1	96.4	98.0	96.3	96.0	94.1	95.8
9.45	97.9	98.5	99.2	99.4	98.5	98.4	97.8	98.2
7.50	99.5	99.7	99.7	99.8	99.5	99.5	99.3	99.4
5.95	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Median diameter (μm)	17.5	14.0	17.0	17.5	27.0	26.0	23.0	28.0

TABLE 3.6 BATCH TO BATCH VARIATION IN SIZE DISTRIBUTION OF NYLON 6.10 AND POLYPHTHALAMIDE MICROCAPSULES, DETERMINED USING A COULTER COUNTER.

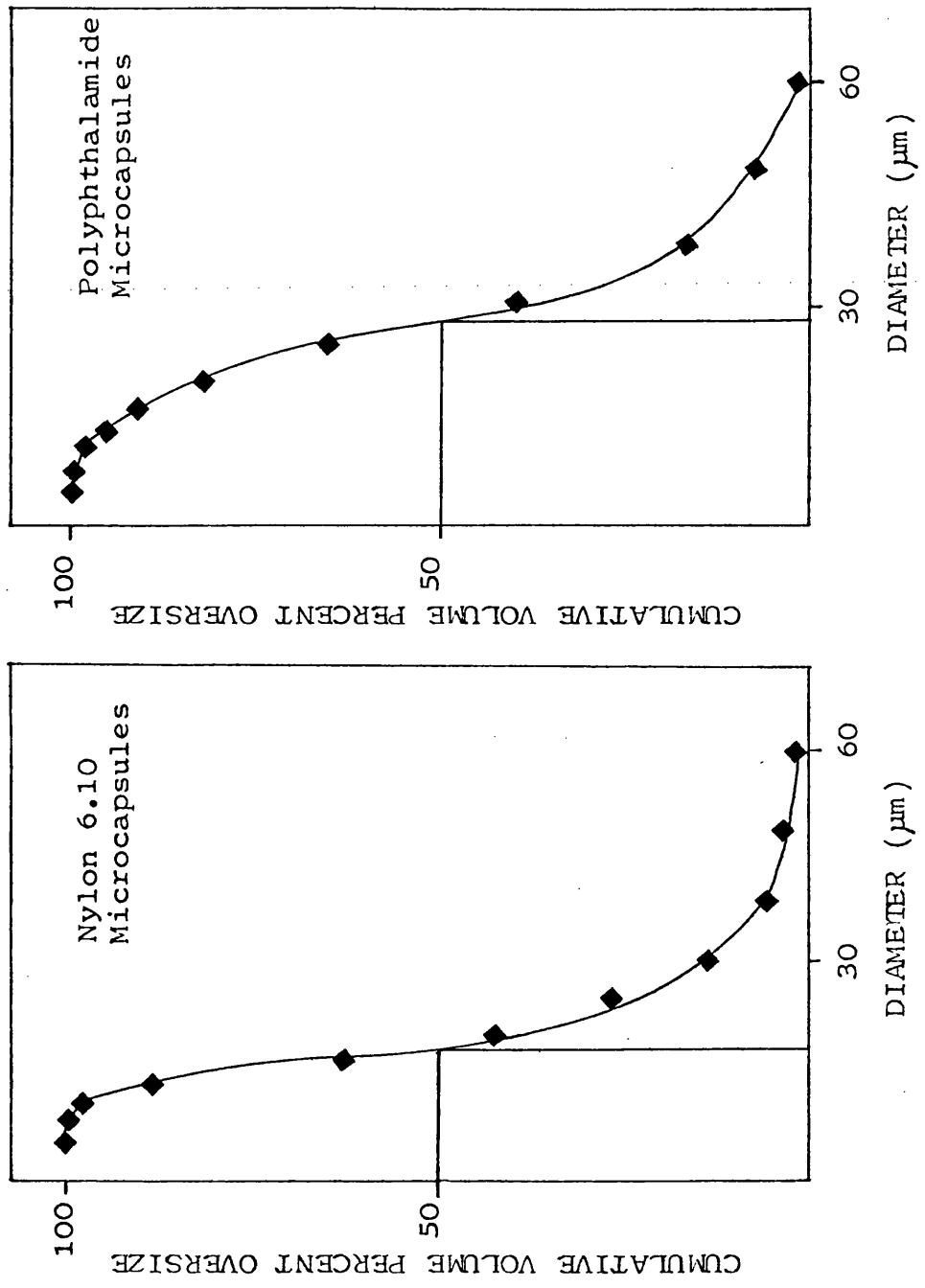


FIGURE 3.7 SIZE DISTRIBUTION OF TYPICAL BATCHES OF NYLON 6.10 AND POLYPHTHALAMIDE MICROCAPSULES.
Determined by Coulter Counter.

Isoton to give a suitable number of microcapsules for measurement and the total capsular volume of this sample was determined by Coulter Counter analysis. Total capsular volume was calculated by the following method:

If the microcapsule diameters fall in the range a-k (μm) and the Coulter Counter size range settings are a-b, b-c, c-d... j-k (μm), then considering the size range a-b, with the number of particles with diameters in that size range equal to N_{ab}, and taking the mean of the size range as the geometric mean

$$dab = \sqrt{a \times b} \qquad \text{(equation 3.1)}$$

the volume of each microcapsule $V_{ab} = {}^{4}/_{3}\pi \left(\frac{dab}{2}\right)^{3}$ (equation 3.2)

then the total volume of microcapsules in the size range

$$= V_{ab} \times N_{ab} \qquad \text{(equation 3.3)}$$

By summing $(V_{ab} \times N_{ab}) + (V_{bc} \times N_{bc}) + \ldots\ldots + (V_{jk} \times N_{jk})$ the total capsular volume in the sample is calculated. Knowing the sample weight and the total capsular volume the concentration of microcapsules in that sample may be expressed as % v/w. If the total slurry weight from which the sample was removed is known then the total capsule volume in the slurry may be calculated. An assessment of the reproducibility of this technique is given in Section 3.4. An example of the calculation of capsule concentration is given for one batch of polyphthalamide microcapsules in Table 3.7. Table 3.8 shows the data obtained for two typical batches of nylon 6.10 microcapsules and four typical batches of polyphthalamide microcapsules. It is evident from Table 3.8 that the yield of polyphthalamide microcapsules is considerably higher than

Size Range. (μm)	Geometric mean of size range. (μm)	Volume of spheres with diameters equal to geometric mean. V (μm^3)	Number of particles with diameters in size range. N	Volume of particles with diameters in size range. V x N (μm^3)
59.98 - 75.58	67.33	159817.5	2	319635
47.61 - 59.97	53.43	79881.6	12	958580
37.78 - 47.60	42.41	39930.2	71	2835047
29.99 - 37.77	33.66	19961.1	310	6187931
23.80 - 29.98	26.71	9979.6	1021	10189133
18.89 - 23.79	21.20	4988.1	1326	6614267
14.99 - 18.88	16.82	2492.9	1301	3243257
11.90 - 14.98	13.35	1246.2	1237	1541546
9.45 - 11.89	10.60	623.6	1294	806963
7.50 - 9.44	8.41	311.9	1210	377428
5.95 - 7.49	6.68	155.8	1029	160292

Total volume of microcapsules in 0.5ml sample of Isoton $= 3.323 \times 10^7 \mu m^3$

Total volume of microcapsules in 200ml Isoton $= 1.329 \times 10^{10} \mu m^3$

Weight of microcapsule slurry dispersed in 200ml Isoton $= 14.61mg$

Therefore, volume of microcapsules in 1g of slurry

$$= \frac{1.329 \times 10^{10}}{0.0146} \mu m^3$$

$$= 0.910 \times 10^{12} \mu m^3$$

Therefore, microcapsule concentration

$$= 91.0\%^{v}/_{w}$$

TABLE 3.7 CALCULATION OF CONCENTRATION OF POLYPHTHALAMIDE MICROCAPSULES (BATCH 1) IN SLURRY FORMED AFTER ROTARY EVAPORATION.

Batch Number	Nylon 6.10		Polyphthalamide			
	1	2	1	2	3	4
Weight of microcapsules after removal from cyclohexane wash.	4.171g	6.296g	9.208g	9.385g	8.935g	9.131g
Weight of microcapsules after rotary evaporation.	2.636g	5.085g	5.026g	5.030g	5.264g	4.422g
Concentration of microcapsules in slurry after rotary evaporation.	8.6 $\%v/_w$	6.6 $\%v/_w$	91.0 $\%v/_w$	109.6 $\%v/_w$	111.2 $\%v/_w$	80.7 $\%v/_w$
Estimated total capsule volume.	0.23ml	0.34ml	4.67ml	5.51ml	5.85ml	3.57ml

TABLE 3.8 YIELD OF NYLON 6.10 AND POLYPHTHALAMIDE MICROCAPSULES.

Batch Number	1	2
Weight of microcapsules after removal from the cyclohexane wash.	5.408g	8.833g
Weight of microcapsules after rotary evaporation.	1.858g	4.138g
Concentration of microcapsules in slurry after rotary evaporation.	27.25%$^{V}/_{W}$	7.7%$^{V}/_{W}$
Estimated total capsular volume.	0.506ml	0.319ml

TABLE 3.9 YIELD OF POLYPHTHALAMIDE MICROCAPSULES PREPARED USING 0.015M PHTHALOYL CHLORIDE.

that of nylon 6.10 microcapsules following rotary evaporation. Polyphthalamide microcapsules prepared using a diacid chloride concentration the same as that used for nylon 6.10 microcapsule preparation, that is 0.015M, have a similar yield to that reported for nylon 6.10 microcapsules (see Table 3.9).

3.3.4 Preparation of Microcapsules in the Presence of Pilocarpine Nitrate

1. Distribution of Pilocarpine Nitrate during Preparation of Microcapsules

Nylon 6.10 and polyphthalamide microcapsules were prepared with pilocarpine nitrate dissolved in the initial aqueous phase for which pilocarpine nitrate concentrations are expressed as the molar concentration of drug present in the initial aqueous phase. Microcapsules were prepared with the aqueous phase containing 1.85×10^{-2}M pilocarpine nitrate. After washing in cyclohexane the microcapsules were washed with 50ml acetone and then dispersed in 150ml of aqueous 5% v/v Tween 20 solution. In order to determine the distribution of the drug during the preparation of the microcapsules, an appropriate volume of stock tritiated pilocarpine solution in water was added to the initial aqueous phase (equivalent to approximately 5×10^5 dpm). Samples of the continuous organic phase formed after quenching, the cyclohexane wash, acetone wash and Tween 20 solution were taken and assayed for pilocarpine nitrate. From a knowledge of the volume of each of these solutions the amount of drug present was calculated and the data are given in Table 3.10. The presence of polyethyleneimine and/or piperazine lowered the count obtained when assaying the initial aqueous phase. Consequently, in order to determine the total activity included in the system the stock

labelled pilocarpine solution was diluted by the same factor as that used to prepare the solution containing polyethyleneimine or piperazine and this assayed for tritiated pilocarpine.

The distribution of the pilocarpine nitrate was also determined in the absence of polymerisation in order to ascertain if the amount of drug found in the organic phase was a function of a partition process between the two phases. Solutions were prepared as for polyphthalamide microcapsule formation containing 1.85 x 10^{-2}M pilocarpine nitrate in the aqueous phase but omitting the phthaloyl chloride and piperazine. 3.75ml 'Solution 1' and 3.75ml 'Solution 2' without piperazine were pipetted into a 250ml conical flask surrounded by ice and stirred at 2,000 rpm. Whilst stirring, 75ml 'Solution 3' was added and stirred for 1 minute after which time 75ml 'Solution 4' without phthaloyl chloride was added and stirred for 3 minutes. The emulsion was poured into 100ml cyclohexane and centrifuged to separate the aqueous and organic phases. Five 200µl samples were removed from each of the initial aqueous phase, the external organic phase and internal aqueous phase and assayed for pilocarpine. The distribution of the pilocarpine nitrate under these conditions is given in Table 3.12.

The data in Tables 3.10 and 3.11 show that during the preparation of both types of microcapsule approximately 50% of the total pilocarpine nitrate recovered was lost to the continuous oil phase, an insignificant amount to the

	Batch 1			Batch 2		
Initial Concentration of Pilocarpine Nitrate in Aqueous Phase.	1.86×10^{-2}M			1.86×10^{-2}M		
Activity of Sample (200µl).	31691 dpm			41893 dpm		
Total count in 3.75ml.	5.94218×10^{5} dpm			7.85502×10^{5} dpm		
	Volume of Phase (ml)	Activity in Total Volume of Phase (dpm)	Fraction of Total Drug Recovered	Volume of Phase (ml)	Activity in Total Volume of Phase (dpm)	Fraction of Total Drug Recovered
Continuous Oil Phase.	235	357763	0.606	236	441993	0.545
Cyclohexane Wash.	49	556	0.001	41	2022	0.003
Acetone Wash.	47	168628	0.286	35	198900	0.245
5% Tween 20 Solution.	160	63150	0.107	164	168620	0.208
Fraction of Initial Mass of Pilocarpine Nitrate Recovered.	0.993			1.033		

TABLE 3.10 THE DISTRIBUTION OF PILOCARPINE NITRATE DURING THE PREPARATION OF NYLON 6.10 MICROCAPSULES.

	Batch 1			Batch 2		
Initial Concentration of Pilocarpine Nitrate in Aqueous Phase.	1.85×10^{-2}M			1.91×10^{-2}M		
Activity of Sample (200µl).	25396 dpm			25992 dpm		
Total Count in 3.75 ml.	4.76181×10^{5} dpm			4.87358×10^{5} dpm		
	Volume of Phase (ml)	Activity in Total Volume of Phase (dpm)	Fraction of Total Drug Recovered	Volume of Phase (ml)	Activity in Total Volume of Phase (dpm)	Fraction of Total Drug Recovered
Continuous Oil Phase.	230	287293	0.558	220	344157	0.654
Cyclohexane Wash.	50	2235	0.004	54	-	-
Acetone Wash.	59	138744	0.269	47	104998	0.199
5% Tween 20 Solution.	152	86505	0.168	150	77475	0.147
Fraction of Initial Mass of Pilocarpine Nitrate Recovered.	0.999			1.000		

TABLE 3.11 THE DISTRIBUTION OF PILOCARPINE NITRATE DURING THE PREPARATION OF POLYPHTHALAMIDE MICROCAPSULES.

	Determination 1			Determination 2		
Initial Concentration of Pilocarpine Nitrate in Aqueous Phase.	1.85×10^{-2}M			1.84×10^{-2}M		
Activity of Sample (200µl).	28678 dpm			21992 dpm		
Total Count in 3.75ml.	5.3771×10^5 dpm			4.1236×10^5 dpm		
	Nominal Volume of Phase (ml)	Activity in Total Volume of Phase (dpm)	Fraction of Total Drug Recovered	Nominal Volume of Phase (ml)	Activity in Total Volume of Phase (dpm)	Fraction of Total Drug Recovered
Continuous Oil Phase.	250	299000	0.534	250	216250	0.561
Discrete Aqueous Phase,	7.5	261192	0.466	7.5	169264	0.439
Fraction of Initial Mass of Pilocarpine Nitrate Recovered.	1.042			0.935		

TABLE 3.12 THE DISTRIBUTION OF PILOCARPINE NITRATE BETWEEN THE DISCRETE AQUEOUS PHASE AND THE CONTINUOUS OIL PHASE IN MICROCAPSULE PREPARATION, IN THE ABSENCE OF POLYMERISATION.

cyclohexane wash and the remainder to the acetone and Tween 20 washes. In the absence of polymerisation (Table 3.12) approximately 50% of the total pilocarpine nitrate recovered was again found in the continuous organic phase suggesting this loss was purely a function of the partition process. From the data in Tables 3.10 and 3.11 it may be expected that if microcapsules were harvested from the cyclohexane wash, that is if the acetone wash and Tween 20 solution were omitted, then the formed microcapsules would contain half the total amount of pilocarpine nitrate included in the system. For this reason, all subsequent preparations of microcapsules were harvested from the cyclohexane wash.

2. The Effect of Pilocarpine Nitrate Concentration on the Preparation of Polyphthalamide Microcapsules

For polyphthalamide microcapsules, the effect of concentration on the loss of pilocarpine nitrate to the organic phase during microcapsule preparation was investigated. Polyphthalamide microcapsules were prepared containing initial pilocarpine nitrate concentrations of 1.85, 3.70, 7.38, 14.76, 18.45 and 29.52×10^{-2}M. The microcapsules were harvested from the cyclohexane wash. After preparation, the organic phase and cyclohexane wash were assayed for pilocarpine nitrate and the appearance of the microcapsules examined after rotary evaporation at 30^{o}C for 30 minutes followed by redispersion in water. The data are given in Table 3.13 and it can be seen that the microcapsules could not be formed in the presence of 18.5×10^{-2}M pilocarpine nitrate

or above. The distribution of the drug in the organic phase was found to be the same for microcapsules containing pilocarpine nitrate at all the lower concentrations. That is approximately 50% of the total mass included in the aqueous phase.

	Initial Concentration of Pilocarpine Nitrate ($M \times 10^2$)					
	1.85	3.69	7.38	14.76	18.45	29.52
Microcapsules formed.	Yes	Yes	Yes	Yes	No	No
Appearance of microcapsules.	Discrete Spheres			Aggre-gates		
Fraction of total pilocarpine nitrate recovered in:-						
1. Continuous organic phase.	0.538	0.586	0.472	0.535		
2. Cyclohexane wash.	0.002	-	0.001	-		

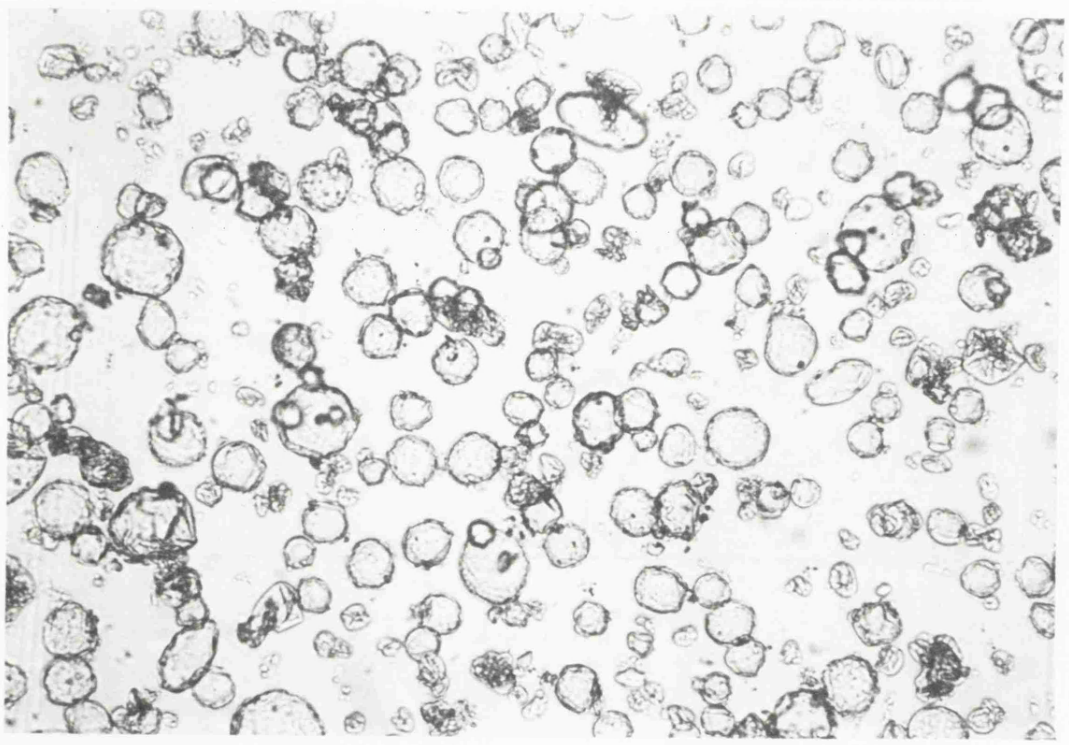
TABLE 3.13 EFFECT OF PILOCARPINE NITRATE CONCENTRATION ON THE FORMATION OF POLYPHTHALAMIDE MICROCAPSULES AND THE DISTRIBUTION OF THE PILOCARPINE NITRATE IN THE PREPARATION.

3.4 Freeze Drying of Polyphthalamide Microcapsules

In an attempt to produce batches of microcapsules of a more uniform nature from which reproducible samples could be removed, microcapsule suspensions were freeze dried. The freeze drier consisted of a large glass vessel at the base of which a flask could be connected. A cold finger protruded into the flask and a vacuum was drawn from the side. Acetone and dry ice mixtures were placed in the cold finger. The sample to be freeze dried was placed in a round bottom flask and frozen by immersing in liquid nitrogen. The flask was attached to the freeze drier and drying continued for a period of approximately 5 hours until the sample reached room temperature.

Freeze drying of polyphthalamide microcapsules resulted in the formation of a fine white free-flowing powder. On addition of acetone to a sample of freeze dried material the microcapsules remained collapsed. On dispersion in water however, they reconstituted well and had a similar appearance to those which had not been freeze dried (Figure 3.8). The scanning electron micrograph in Figure 3.9a also shows the microcapsule has reconstituted and is hollow. The thickness of the wall is estimated to be approximately 0.8μm. The micrograph in Figure 3.9b however suggests that some of the microcapsules have not fully reconstituted as these have remained collapsed.

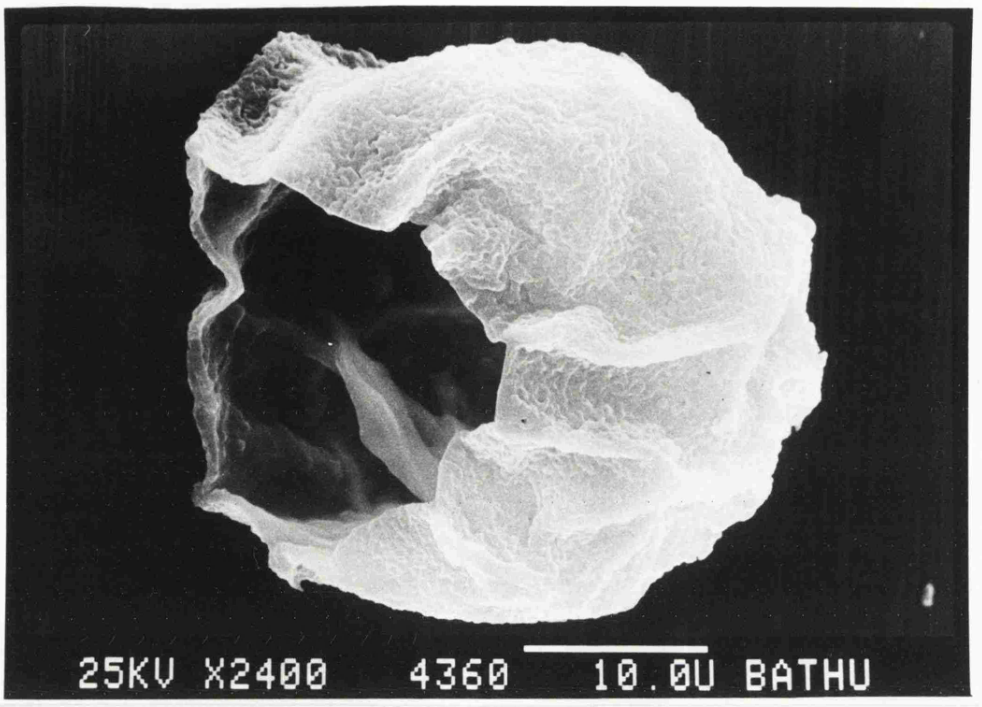
The effect of freeze drying on the size distribution of polyphthalamide microcapsules is shown in Figure 3.10 which gives the size distribution of 2 batches of polyphthalamide microcapsules before and after freeze drying. The curves for the samples of freeze dried and non freeze dried material almost superimpose.



Scale: ⊢——⊣ 50μm

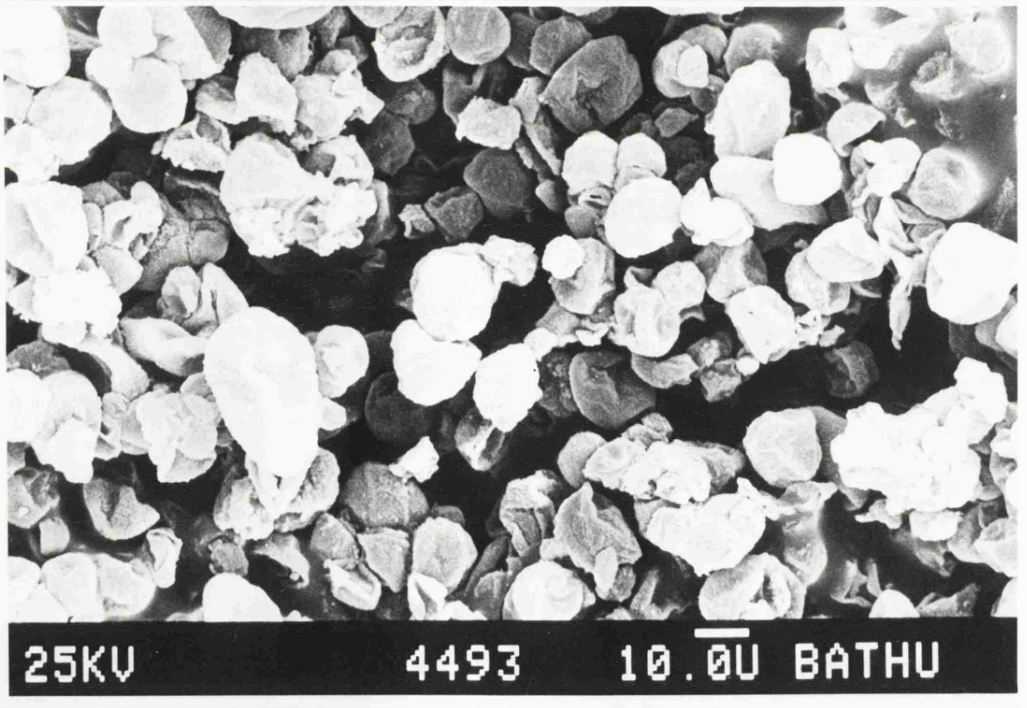
FIGURE 3.8 FREEZE DRIED POLYPHTHALAMIDE MICROCAPSULES AFTER DISPERSION IN WATER.

a.



Scale: 10.0μm

b.



Scale: 20.0μm

FIGURE 3.9 SCANNING ELECTRON MICROGRAPHS OF FREEZE DRIED POLYPHTHALAMIDE MICROCAPSULES.

a. Prepared by freeze fracture method
b. Prepared by critical point drying method

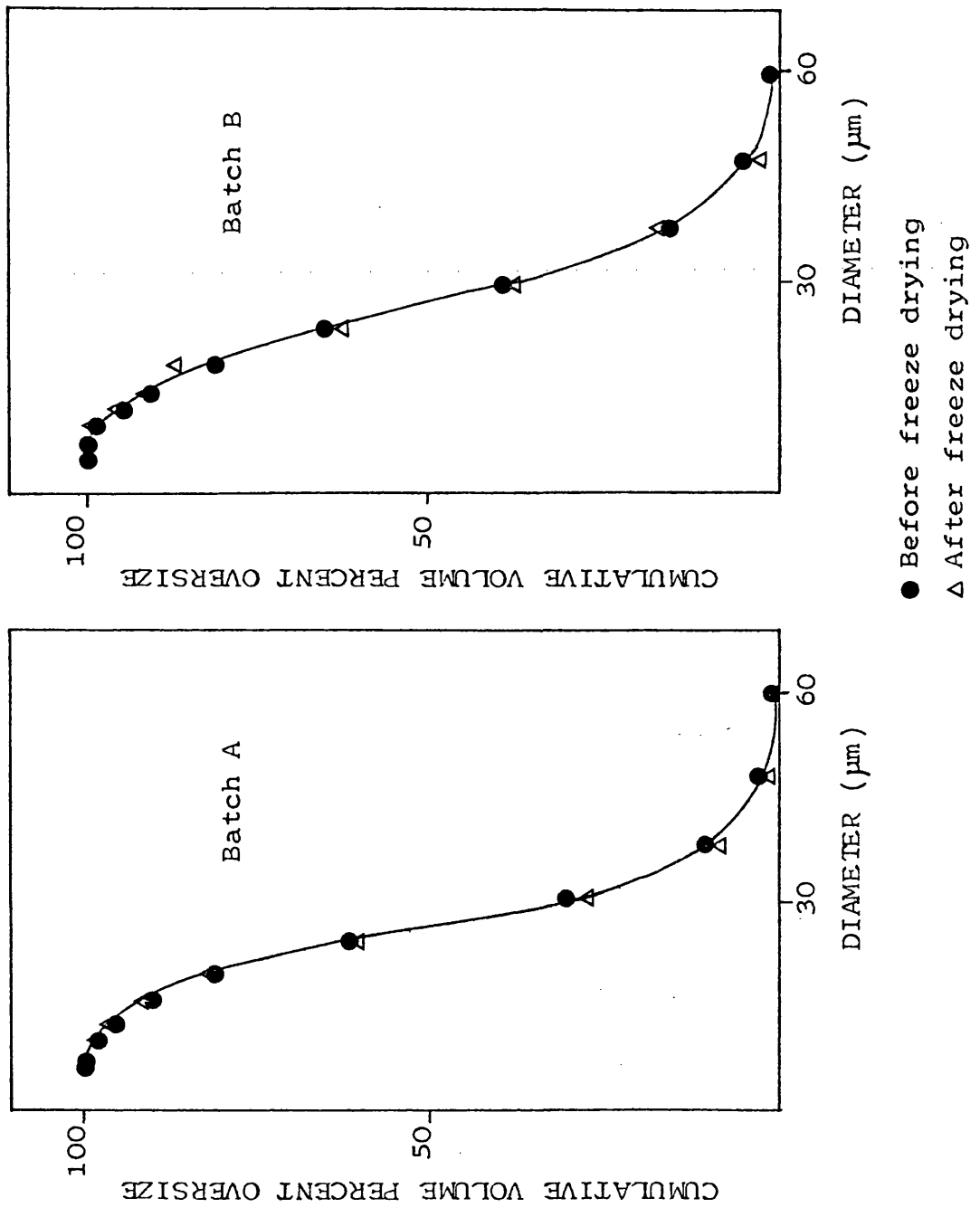


FIGURE 3.10 THE EFFECT OF FREEZE DRYING AND RECONSTITUTION IN WATER ON THE SIZE DISTRIBUTION OF POLYPHTHALAMIDE MICROCAPSULES.

The yield of microcapsules obtained following freeze drying was also determined using the Coulter Counter. In order to assess the reproducibility of this method for determining the concentration of freeze dried microcapsules, five samples of known weight were taken from a given batch of freeze dried polyphthalamide microcapsules and their 'concentrations' determined as given in Section 3.3.3 following reconstitution in a known volume of Isoton. The percent volume in weight determined for each sample is given in Table 3.14. The 'concentration' values are in good agreement and have a coefficient of variation of ± 2.7%. Data for the yield of microcapsules following freeze drying of five different batches of polyphthalamide microcapsules are given in Table 3.15. The concentration of microcapsules in the freeze dried powder, determined by Coulter Counter as in Section 3.3.3 was found to range between approximately 200 and 300% v/w for four batches. The reproducibility of this method of determining capsule concentration suggests that the large variation in concentration is due to a difference in the microcapsule batches and does not arise as a result of the method of measurement used.

Sample	Weight of microcapsules taken (mg)	Estimated concentration $\%^{V}/_{W}$	Standard deviation of concentration	Coefficient of variation
1	1.93	187.6		
2	3.82	189.8		
3	4.99	199.6	5.276	± 2.7%
4	5.81	195.9		
5	7.29	188.2		

TABLE 3.14 REPRODUCIBILITY OF THE DETERMINATION OF MICROCAPSULE CONCENTRATION BY COULTER COUNTER.

Batch	1	2	3	4
Weight of microcapsules after removal from cyclohexane wash	9.208g	9.385g	8.935g	9.131g
Weight of microcapsules after rotary evaporation.	5.026g	5.030g	5.264g	4.422g
Weight of microcapsules after freeze drying.	1.769g	1.021g	1.076g	2.017g
Concentration of microcapsules after freeze drying.	253.0 $\%v/_w$	306.6 $\%v/_w$	290.7 $\%v/_w$	213.3 $\%v/_w$

TABLE 3.15 EFFECT OF FREEZE DRYING ON THE YIELD OF POLYPHTHALAMIDE MICROCAPSULES.

3.5 Release from Polyphthalamide Microcapsules

3.5.1 Release of Pilocarpine Nitrate from Polyphthalamide Microcapsules

3.5.1.1 General Method

The release of pilocarpine nitrate from polyphthalamide microcapsules was determined by the following method. A known weight of microcapsule slurry or freeze dried powder containing the drug was accurately weighed into a 50ml conical flask fitted with a glass stopper. To this was added 50ml isotonic phosphate buffer equilibrated to 32°C. The flask was then shaken in a waterbath at 70 cycles per minute and 32°C, ensuring that the level of water in the bath was above that of the suspension in the flask. Approximately 1.5ml samples of the solution were removed at timed intervals through a 3μm Millipore filter connected to a syringe. The samples were weighed after removal and assayed for pilocarpine nitrate. In a separate experiment to assess the uptake of pilocarpine nitrate onto Millipore filters, five samples were removed from a stock solution of pilocarpine nitrate also containing labelled pilocarpine. Each was filtered through a clean Millipore filter. No significant difference in activity before and after passing through the filter was observed. Throughout the determination of release the volumes of solution taken for sampling (that is 1.5ml) were significant relative to the original volume of solution present. It was therefore necessary to correct for the amount of pilocarpine nitrate removed in each sample in calculating the total mass released.

3.5.1.2 Determination of Release of Pilocarpine Nitrate

The release of pilocarpine nitrate was determined from four different types of polyphthalamide microcapsule preparation. The techniques used are given below.

Method 1

Microcapsules were prepared containing a known concentration of pilocarpine nitrate together with an aliquot of tritiated pilocarpine stock solution in the aqueous phase. After washing in cyclohexane the microcapsule slurry was rotary evaporated for 30 minutes at 30°C. Approximately 5.0g of the slurry was accurately weighed into a 50ml conical flask and the release measured according to the general method (see Section 3.5.1.1).

Method 2

This technique involved freeze drying polyphthalamide microcapsules prepared in the absence of pilocarpine nitrate, which were then resuspended in pilocarpine nitrate solution containing tritiated pilocarpine. The release from these reconstituted microcapsules now containing the drug was determined by the addition of a further volume of buffer solution. Polyphthalamide microcapsules containing electrolyte solution were prepared as in Section 3.2 and frozen in liquid nitrogen. After freeze drying for 5 hours approximately 1.5g of the dried material,accurately weighed,was immersed in 5ml of a solution of pilocarpine nitrate and tritiated pilocarpine in pH7.4 isotonic phosphate buffer to give a thick suspension. The suspension was allowed to

equilibrate at 32°C for 12 hours. A volume of pH7.4 isotonic buffer at 32°C sufficient to give 50ml was then added and the release determined as given in the general method (see Section 3.5.1.1).

In order to ensure that the pilocarpine nitrate penetrated the freeze dried capsule wall on resuspension, it was necessary to determine the interaction of pilocarpine nitrate with the intact microcapsules and with the polyphthalamide wall material. After preparation, polyphthalamide microcapsules were washed with cyclohexane, rotary evaporated and dispersed in water before freeze drying for 5 hours. Approximately 0.7g freeze dried capsules accurately weighed, was placed in a flask to which was added 5ml 1.47×10^{-1}M pilocarpine nitrate in pH 7.4 isotonic phosphate buffer containing tritiated pilocarpine. Five 20µl samples were removed through a Millipore filter and assayed for pilocarpine nitrate. For the wall material, polyphthalamide microcapsules were prepared as in Section 3.2 and after washing with cyclohexane the slurry was rotary evaporated and dispersed in water. The suspension was ultrasonicated for 1 hour, centrifuged and the microcapsule walls freeze dried. Measurement of the uptake of pilocarpine nitrate was as for the freeze dried intact microcapsules. The uptake of pilocarpine nitrate by whole and broken microcapsules is given in Table 3.16 and the data show that the uptake of pilocarpine nitrate per gram of freeze dried material was not significantly different for the polyphthalamide microcapsules and the broken walls. It is probable therefore that the pilocarpine nitrate penetrates the microcapsule wall and gains access to the aqueous core of the microcapsules.

Sample	Weight of Sample (g)	Initial Pilocarpine Nitrate Concentration ($M \times 10^4$)	Final Pilocarpine Nitrate Concentration ($M \times 10^4$)	Uptake of Pilocarpine Nitrate per gram of Sample ($M\ g^{-1} \times 10^4$)
Intact Microcapsules.				
A	0.7335	1.478	1.264	1.580
B	0.6814	1.478	1.261	1.591
Polyphthalamide Wall Material.				
A	0.6656	1.470	1.287	1.378
B	0.7292	1.472	1.270	1.387

TABLE 3.16 UPTAKE OF PILOCARPINE NITRATE BY FREEZE DRIED POLYPHTHALAMIDE MICROCAPSULES AND POLYPHTHALAMIDE MICROCAPSULE WALL MATERIAL.

Method 3

Polyphthalamide microcapsules were prepared containing pilocarpine nitrate and an aliquot of tritiated pilocarpine solution in the aqueous phase. After washing in cyclohexane the slurry was frozen in liquid nitrogen and freeze dried for 5 hours. Approximately 1.0g of the freeze dried powder was accurately weighed into a 50ml conical flask and the release measured according to the general method (see Section 3.5.1.1).

Method 4

The measurement of release by 'Method 4' was similar to that described for 'Method 3'. Microcapsules containing pilocarpine nitrate were washed in cyclohexane and frozen in liquid nitrogen. They were then freeze dried but for a period of only 30 minutes which resulted in a slurry rather than a powder. Approximately 4.0g of the slurry was accurately weighed into a 50ml conical flask and the release measured as given in Section 3.5.1.1. In this instance the samples were assayed by HPLC. For the purpose of washing the loop valve injector of the HPLC system and the syringe used for injection onto the column it was necessary to remove 3.0ml volume samples and therefore fewer samples were taken. In calculating the mass released it was again necessary to correct for the mass of pilocarpine nitrate present in each sample removed.

3.5.1.3 Release Profiles

The release of pilocarpine nitrate from polyphthalamide microcapsules determined by the four methods described above is shown in Figures 3.11 to 3.14. Figure 3.11 and the data given in Tables 3.17 and 3.18 show the effect of concentration on the release of pilocarpine nitrate over the concentration range 1.85×10^{-2}M to 14.78×10^{-2}M determined by 'Method 1' and indicate that the rate of release of pilocarpine nitrate is dependent upon initial drug concentration. It is apparent that more than 90% of the total pilocarpine nitrate content recovered from the microcapsule phase of initial concentrations approximately 1.85×10^{-2}M and 3.61×10^{-2}M was released in 20 to 30 minutes. However, although the microcapsules containing 7.36×10^{-2}M and 14.78×10^{-2}M pilocarpine nitrate lost approximately 50% of the total drug content recovered in the first minute the remainder was lost considerably more slowly. Figure 3.11 also shows that there was a 10 to 20% variation in the total mass of pilocarpine nitrate released between replicate determinations for a given initial drug concentration. This arises due to the following factors:

1. during microcapsule preparation the amount of drug lost to the organic phase is variable (see Section 3.3.4) and therefore the microcapsule phase does not contain a constant mass of pilocarpine nitrate.
2. the weight of microcapsules formed is not constant and therefore the final total weight of drug per gram of microcapsule slurry alters (see Section 3.3.3).

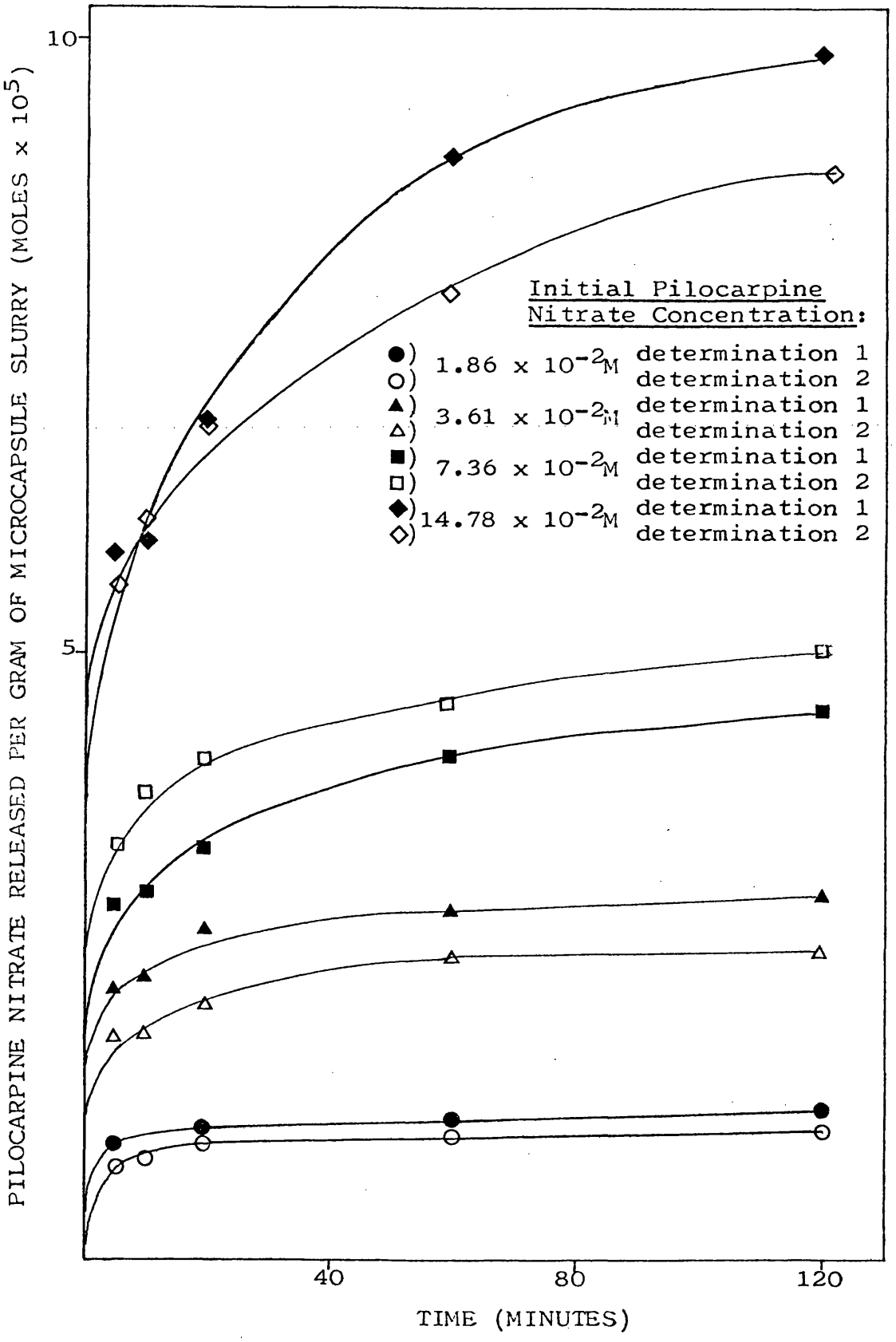


FIGURE 3.11 THE EFFECT OF CONCENTRATION ON THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C AS DETERMINED USING 'METHOD 1' SECTION 3.5.1.2.

Initial Pilocarpine Nitrate Concentration.	1.86×10^{-2}M	1.85×10^{-2}M	3.61×10^{-2}M	3.61×10^{-2}M
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.395×10^{-4} moles	1.388×10^{-4} moles	2.708×10^{-4} moles	2.708×10^{-4} moles
Fraction of Total Mass of Pilocarpine Nitrate Included, Recovered in Organic Phase and Cyclohexane Wash.	0.573	0.507	0.560	0.611
Sample Weight taken from Microcapsule Slurry (≡ to total mass formed).	5.026g	5.370g	4.892g	5.056g
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals. Time (minutes)	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.
5	1.009 82.2	0.795 73.7	2.260 75.1	1.816 71.1
10	1.043 84.9	0.858 79.7	2.359 78.2	1.851 73.4
20	1.102 89.7	0.985 91.3	2.825 93.6	2.120 85.3
60	1.198 97.6	1.034 96.0	2.854 94.6	2.514 99.3
120	1.228 100.0	1.078 100.0	3.017 100.0	2.532 100.0
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.442	0.417	0.545	0.472

TABLE 3.17 THE EFFECT OF CONCENTRATION ON THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 1'.

Initial Pilocarpine Nitrate Concentration.	7.36×10^{-2}M	7.36×10^{-2}M	14.78×10^{-2}M	14.78×10^{-2}M
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	5.552×10^{-4} moles	5.552×10^{-4} moles	11.09×10^{-4} moles	11.09×10^{-4} moles
Fraction of Total Mass of Pilocarpine Nitrate Included, Recovered in Organic Phase and Cyclohexane Wash.	0.502	0.542	2.423	0.544
Sample Weight taken from Microcapsule Slurry (≡ to total mass formed).	5.008g	5.132g	6.850g	6.721g
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals. Time (minutes)	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Slurry. x 10^5 / Percent of Total Mass Released in 120 Minutes.
5	3.446 67.3	2.967 64.1	5.650 62.7	5.881 58.9
10	3.886 75.9	3.046 65.8	6.241 69.3	5.932 59.4
20	4.173 81.5	3.435 74.2	6.972 77.4	6.879 68.9
60	4.605 89.9	4.162 89.9	7.971 88.5	9.126 91.4
120	5.132 100.0	4.650 100 0	9.007 100.0	9.985 100.0
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.462	0.428	0.556	0.605

TABLE 3.18 THE EFFECT OF CONCENTRATION ON THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 1'.

3. for a given weight of microcapsule slurry formed the microcapsule 'concentration' in the slurry is changeable (see Section 3.3.3) and therefore in sampling a given weight of slurry a variable weight of microcapsules and hence an inconstant weight of drug is taken.

The absolute values of release rate in terms of moles of drug released per gram of microcapsule slurry at given time intervals cannot be compared for two different methods of determination due to the difference in the physical nature of the microcapsule slurry or powder sampled and the relative microcapsule concentrations. The release rates in terms of moles of drug released per batch of microcapsules also cannot be compared due to variable unknown microcapsule losses during drying for example. For this reason, together with the changeable mass of drug encapsulated for a given initial drug concentration, release rates are discussed in terms of percent total pilocarpine nitrate released in 120 minutes. To ensure equilibrium had been reached within 120 minutes some determinations were continued for 24 hours. Alternatively, data for the fraction of initial pilocarpine nitrate content recovered in the external organic phase, the cyclohexane wash and the release medium are included in Tables 3.17 and 3.18 to show that within the accuracy of the experiment 100% of the total mass included was recovered.

Figure 3.12 and Table 3.19 show the release of pilocarpine nitrate from polyphthalamide microcapsules determined by 'Method 2'. Again there is some variation in the profiles for replicate determinations of the same initial pilocarpine nitrate content. In this instance the variation is due only

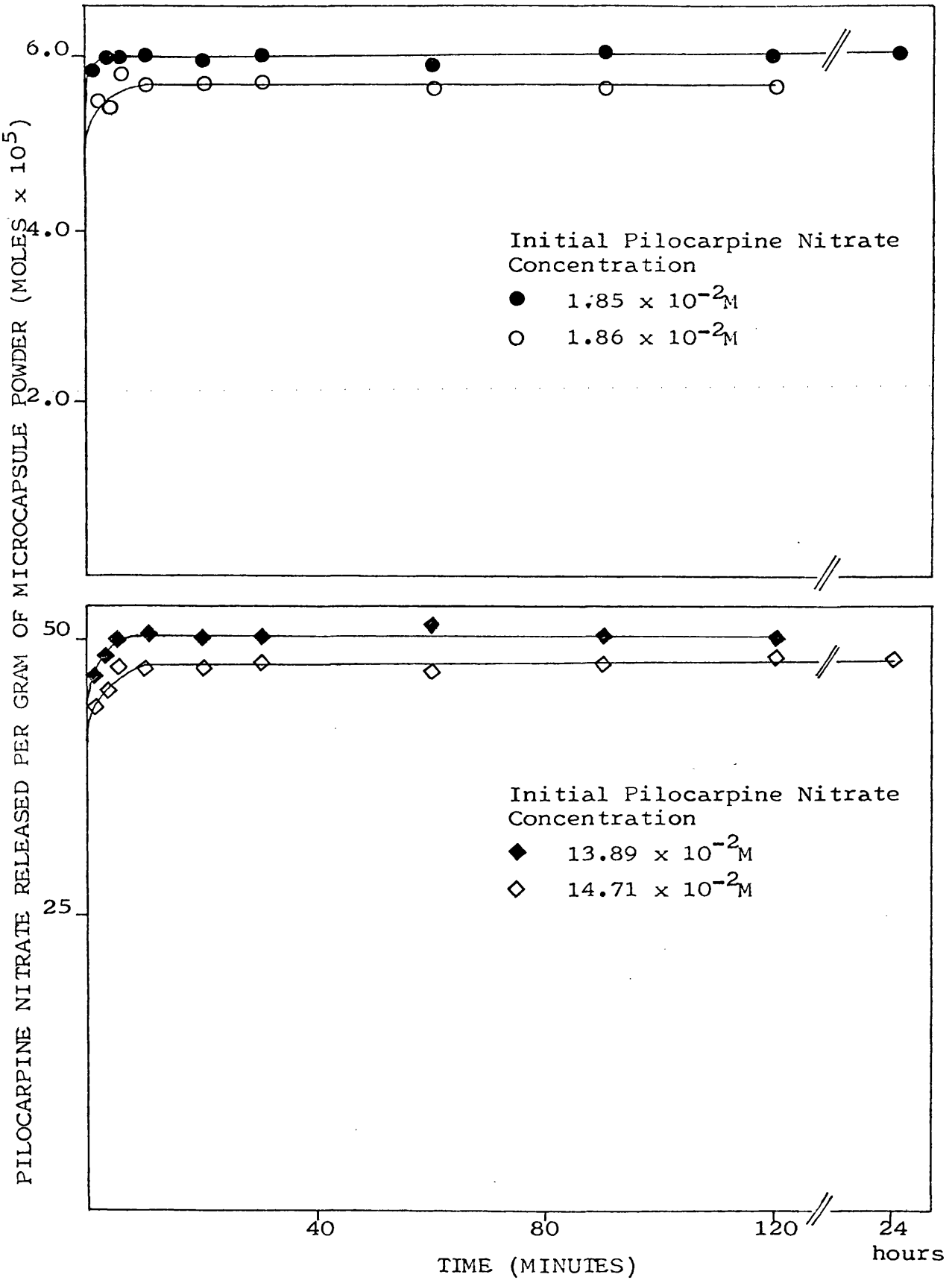


FIGURE 3.12 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED BY 'METHOD 2'.

Initial Pilocarpine Nitrate Concentration.	1.85×10^{-2}M	1.86×10^{-2}M	13.89×10^{-2}M	14.72×10^{-2}M
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	0.923×10^{-4} moles	0.929×10^{-4} moles	6.945×10^{-4} moles	7.358×10^{-4} moles
Sample Weight taken from Dried Microcapsule Powder.	1.4397g	1.5530g	1.4114g	1.5303g

Relase of Pilocarpine Nitrate from Microcapsules at Timed Intervals. Time (minutes)	Moles Released per gram of Microcapsule Powder. x 10^5	Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Powder. x 10^5	Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Powder. x 10^5	Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Powder. x 10^5	Percent of Total Mass Released in 120 Minutes.
1	5.911	97.6	5.549	97.2	46.70	92.1	44.32	90.7
3	6.024	99.5	5.473	95.9	48.92	96.5	45.44	93.0
5	6.061	100.1	5.840	102.3	50.41	99.4	47.99	98.2
10	6.113	100.9	5.743	100.6	50.78	100.2	47.83	97.9
20	6.062	100.1	5.775	101.2	50.35	99.3	47.76	97.8
30	6.100	100.7	5.725	100.3	50.64	99.9	48.07	98.4
60	5.955	98.3	5.655	99.1	51.76	102.1	47.10	96.4
90	6.170	101.9	5.673	99.4	50.63	99.9	48.08	98.4
120	6.057	100.0	5.708	100.0	50.69	100.0	48.85	100.0
24 hours	6.140	101.4	-	-	-	-	48.44	99.2
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.957		0.954		1.030		1.007	

TABLE 3.19 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 2'.

to a difference in the mass of freeze dried microcapsules sampled. The release of the drug was again extremely rapid and the rate of release was dependent upon initial pilocarpine nitrate concentration. At both initial concentrations investigated, however, approximately 99% of the total mass of drug released was lost within the first five minutes.

The release of pilocarpine nitrate from polyphthalamide microcapsules as determined by 'Method 3' is given in Figure 3.13 and Table 3.20 which again show pilocarpine nitrate release to be rapid. Approximately 50% of the pilocarpine nitrate recovered from the microcapsules was released during the first minute although the remainder was lost more slowly from the microcapsules containing the higher initial starting concentration of the drug. For example, the microcapsules prepared containing approximately 1.85×10^{-2}M pilocarpine nitrate lost 90% of the total amount of drug released within the first five minutes. In the case of the microcapsules prepared containing approximately 14.7×10^{-2}M pilocarpine nitrate a similar percentage was not released until after approximately 20 minutes. It is apparent from Figure 3.13 that there is a large difference in the total mass of pilocarpine nitrate released per gram of the two batches of microcapsules prepared containing approximately 1.85×10^{-2}M pilocarpine nitrate. This is believed to be due to the large variation in microcapsule concentration observed for freeze dried material (see Section 3.4). That is, in sampling 1g of freeze dried powder the number of microcapsules present varies and consequently the total mass of pilocarpine nitrate present changes.

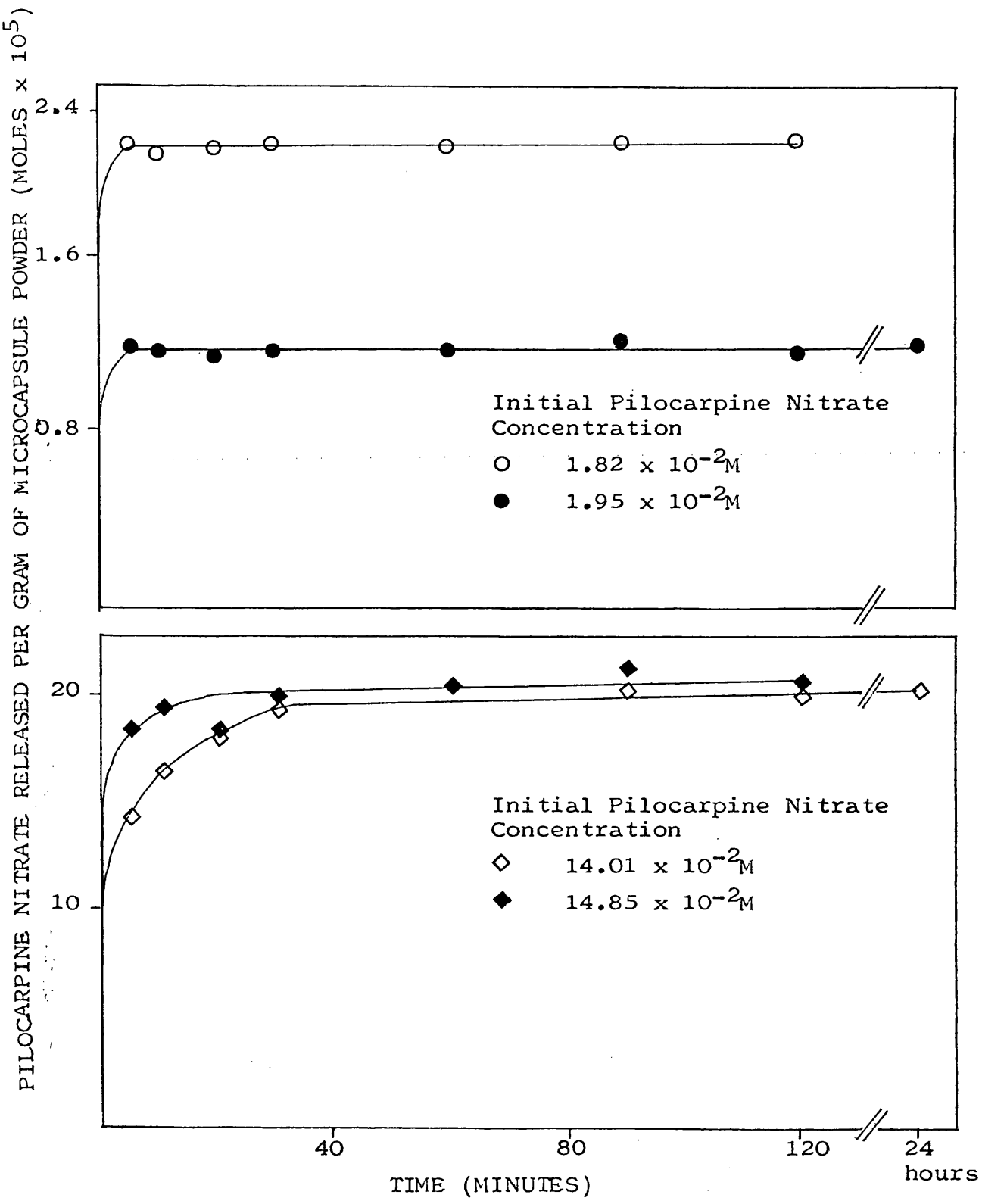


FIGURE 3.13 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED BY 'METHOD 3'.

Initial Pilocarpine Nitrate Concentration.	1.95×10^{-2}M	1.82×10^{-2}M	14.85×10^{-2}M	14.01×10^{-2}M
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.462×10^{-4} moles	1.365×10^{-4} moles	11.14×10^{-4} moles	10.51 x 10^{-4} moles
Sample Weight taken from dried microcapsule powder.	1.4651g	1.4772g	1.4917g	1.6258g
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals. Time (minutes)	Moles Released per gram of Microcapsule Powder. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Powder. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Powder. x 10^5 / Percent of Total Mass Released in 120 Minutes.	Moles Released per gram of Microcapsule Powder. x 10^5 / Percent of Total Mass Released in 120 Minutes.
5	1.184 / 102.8	2.130 / 99.2	18.64 / 89.1	14.41 / 71.9
10	1.164 / 101.4	2.064 / 96.1	19.47 / 93.0	16.56 / 82.6
20	1.139 / 98.9	2.092 / 97.4	18.65 / 89.1	18.45 / 92.1
30	1.169 / 101.5	2.128 / 99.1	20.27 / 96.8	19.51 / 97.4
60	1.169 / 101.5	2.113 / 98.4	21.52 / 98.0	17.62 / 87.9
90	1.202 / 104.3	2.134 / 99.4	21.38 / 102.2	20.57 / 102.6
120	1.152 / 100.0	2.147 / 100.0	20.93 / 100.0	20.04 / 100.0
24 hours	1.221 / 106.0	-	-	20.48 / 102.2
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.115	0.232	0.280	0.310

TABLE 3.20 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 3'.

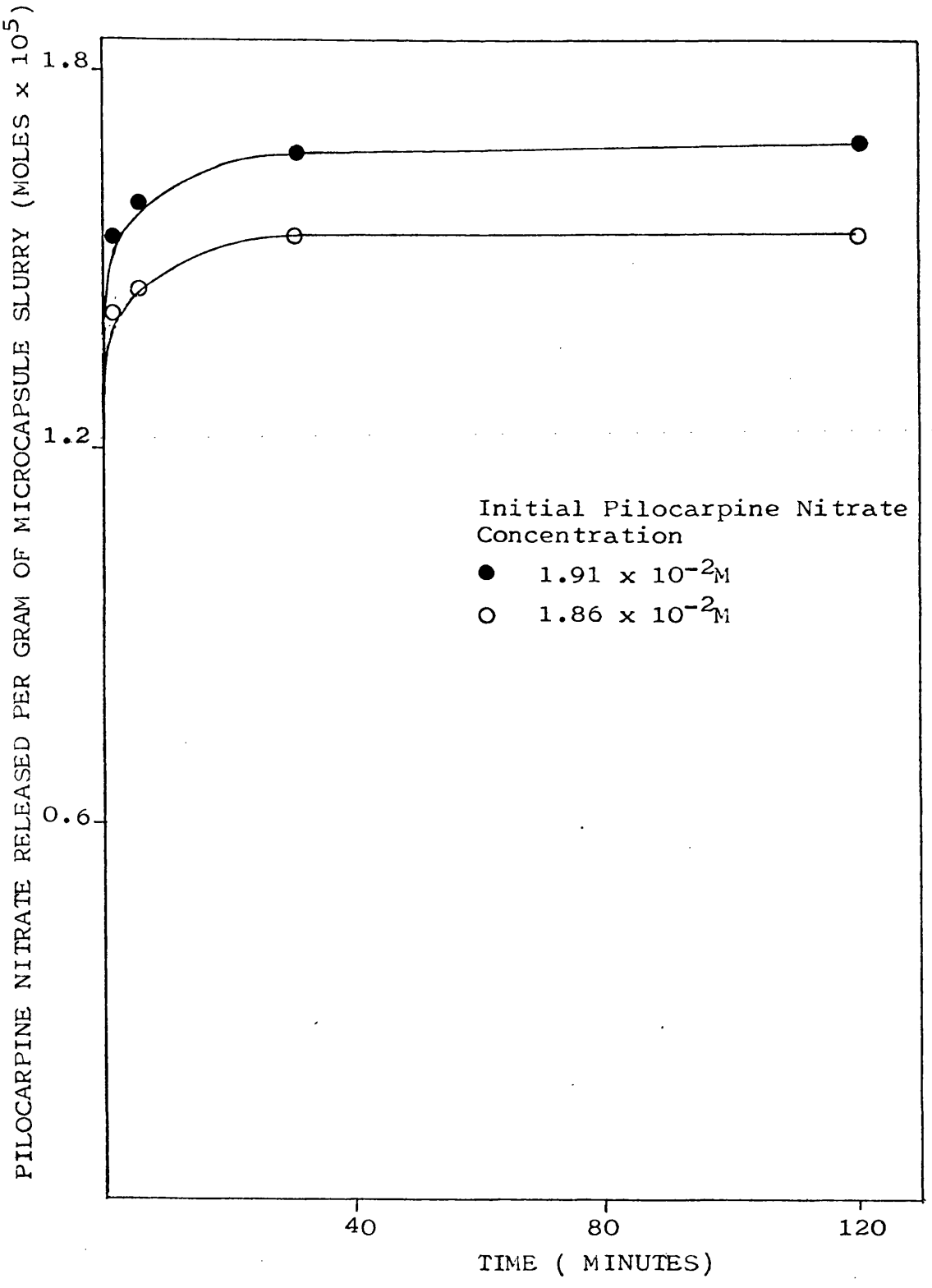


FIGURE 3.14 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED BY 'METHOD 4'.

Initial Pilocarpine Nitrate Concentration.	1.91×10^{-2}M		1.86×10^{-2}M	
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.429×10^{-4} moles		1.395×10^{-4} moles	
Sample Weight taken from Microcapsule Slurry.	4.6021g		5.0482g	
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals.	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes. %	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes. %
Time (minutes)				
1	1.525	92.4	1.425	92.8
5	1.591	94.8	1.458	95.0
30	1.671	99.6	1.538	100.3
120	1.678	100.0	1.534	100.0
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.540		0.555	

TABLE 3.21 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 4'.

The release of pilocarpine nitrate determined by 'Method 4' is given in Figure 3.14 and Table 3.21 from which it may be seen that the drug was again released rapidly. Within the first minute 90% of the total pilocarpine nitrate recovered from the microcapsule phase was released. This method was used to determine the release from microcapsules containing 1.85×10^{-2}M pilocarpine nitrate only.

3.5.2 Degradation of Pilocarpine Nitrate During Microcapsule Preparation and Release

To determine the extent of pilocarpine nitrate degradation during microcapsule preparation and subsequent release, the release medium sampled in 'Method 4' above was analysed by HPLC. The conditions were as for pilocarpine nitrate assay by HPLC except that the sensitivity was increased to 0.04 aufs. The chromatogram obtained is shown in Figure 3.15. No peaks corresponding to isopilocarpine, pilocarpic acid or isopilocarpic acid were observed, although the shoulder on the pilocarpine nitrate peak may suggest the presence of trace amounts of pilocarpic acid. The peaks present corresponding to 2 minutes 6 seconds and 4 minutes 45 seconds are believed to be impurities present in the microcapsule system such as unreacted monomers.

3.5.3 Release of Prednisolone Sodium Phosphate from Polyphthalamide Microcapsules

Polyphthalamide microcapsules were prepared containing 1.86×10^{-2}M prednisolone sodium phosphate by dissolving the drug in the internal aqueous phase prior to encapsulation. After washing in cyclohexane the microcapsule slurry was frozen in liquid nitrogen and freeze dried for 30 minutes. Measurement of the

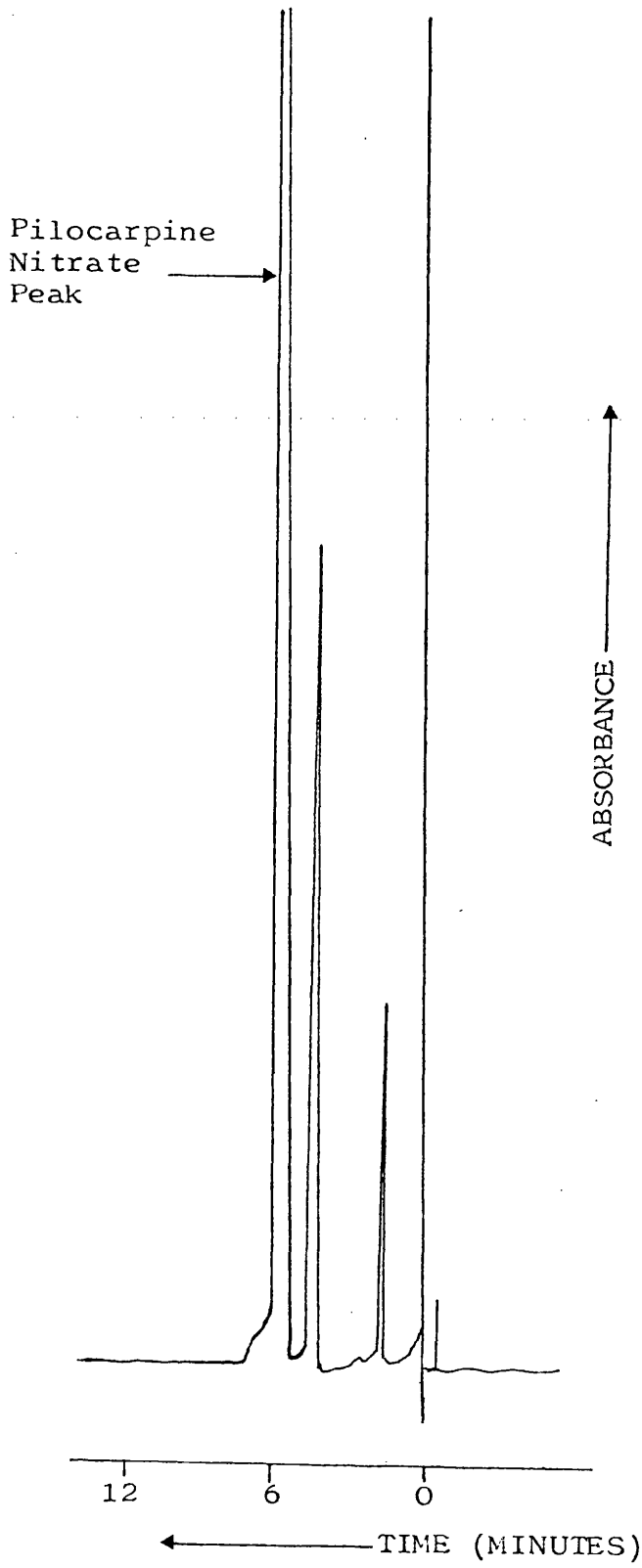


FIGURE 3.15 HPLC TRACE SHOWING IMPURITIES PRESENT IN SOLUTION AFTER THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES.

release of prednisolone sodium phosphate was determined using 'Method 4' as given in Section 3.5.1.2 for pilocarpine nitrate. Figure 3.16 shows a plot of the moles of prednisolone sodium phosphate released per gram of microcapsule slurry against time from which it may be seen that the release of the prednisolone sodium phosphate was rapid. Approximately 60% of the drug content released in 120 minutes was lost in the first minute, and 90% within the first 15 minutes. Data for the release profile are given in Table 3.22.

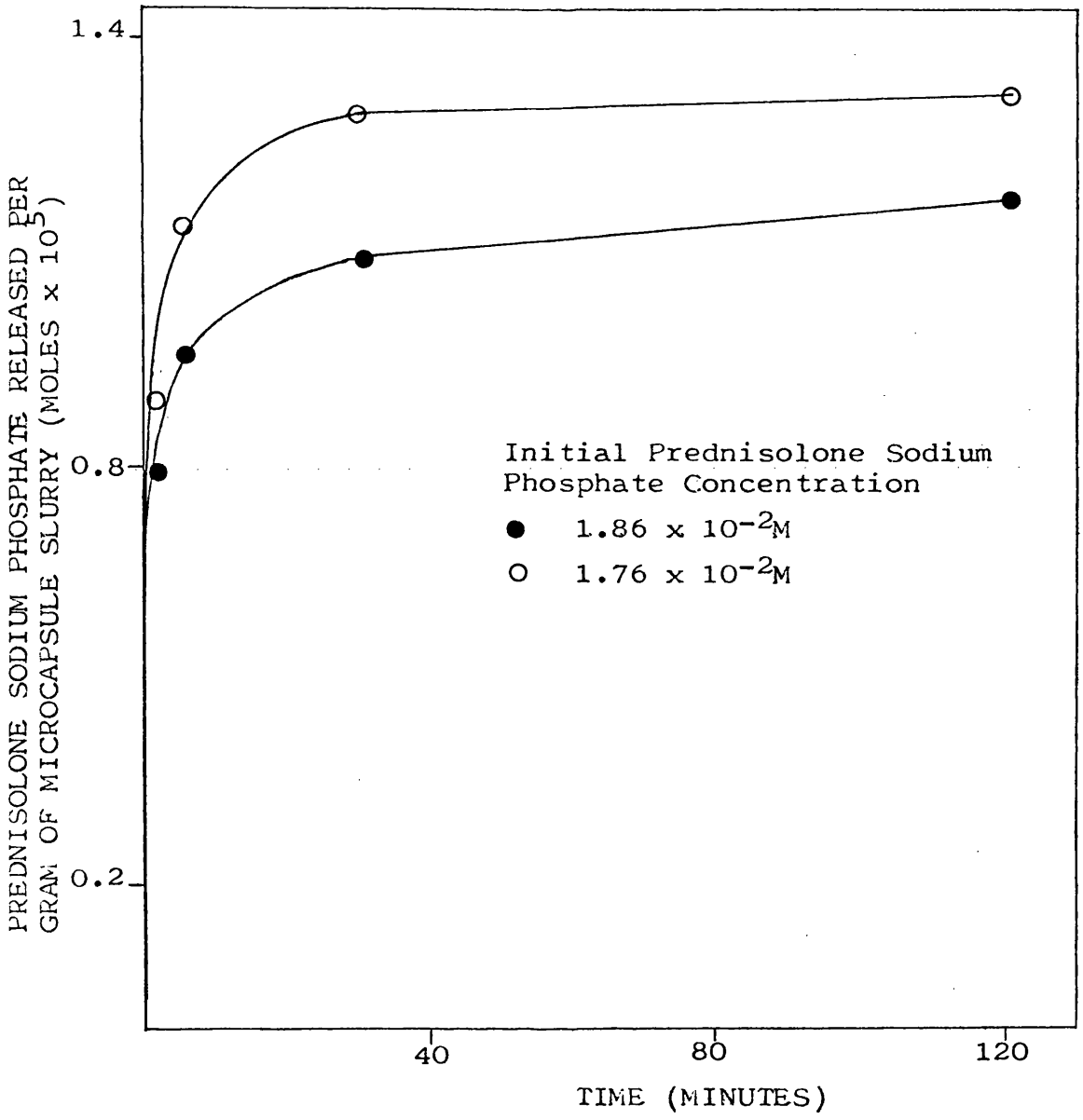


FIGURE 3.16 THE RELEASE OF PREDNISOLONE SODIUM PHOSPHATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 4'.

Initial Prednisolone Sodium Phosphate Concentration.	1.86×10^{-2}M		1.76×10^{-2}M	
Mass of Prednisolone Sodium Phosphate in Initial Aqueous Phase.	1.397×10^{-4} moles		1.323×10^{-4} moles	
Sample Weight taken from Microcapsule Slurry.	6.6479g		6.3549g	
Release of Prednisolone Sodium Phosphate from Microcapsules at Timed Intervals.	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes. %	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes. %
Time (minutes)				
1	0.786	67.3	0.898	67.9
5	0.953	81.7	1.137	86.0
30	1.086	93.1	1.298	98.2
120	1.167	100.0	1.322	100.0
24 hours	1.237	105.9	-	-
Mass of Prednisolone Sodium Phosphate Released Expressed as Fraction of Total Mass Included.	0.589		0.635	

TABLE 3.22 THE RELEASE OF PREDNISOLONE SODIUM PHOSPHATE FROM POLYPHTHALAMIDE MICROCAPSULES INTO pH 7.4 BUFFER AT 32°C DETERMINED USING 'METHOD 4'.

3.6 Polyphthalamide Microcapsules - Modifications to Core and Wall. Preparation and Characteristics

Polyphthalamide microcapsules were prepared with a modified core or modified walls by methods based on that outlined in Section 3.2. For each type of microcapsule with the exception of the albumin containing microcapsules the following characteristics were investigated. The appearance of microcapsules was examined microscopically following freezing of microcapsule suspensions in liquid nitrogen and freeze drying for 30 minutes before resuspending in water and mounting in a haemocytometer. The size distribution of microcapsule suspensions prepared as above was determined using a Coulter Counter and the release of pilocarpine nitrate was measured using 'Method 4'. For this, microcapsules were prepared containing approximately 1.9×10^{-2}M pilocarpine nitrate in the aqueous phase together with tritiated pilocarpine. The assay technique used was liquid scintillation counting.

3.6.1 Polyphthalamide Microcapsules containing Gelatin and Crosslinked Gelatin

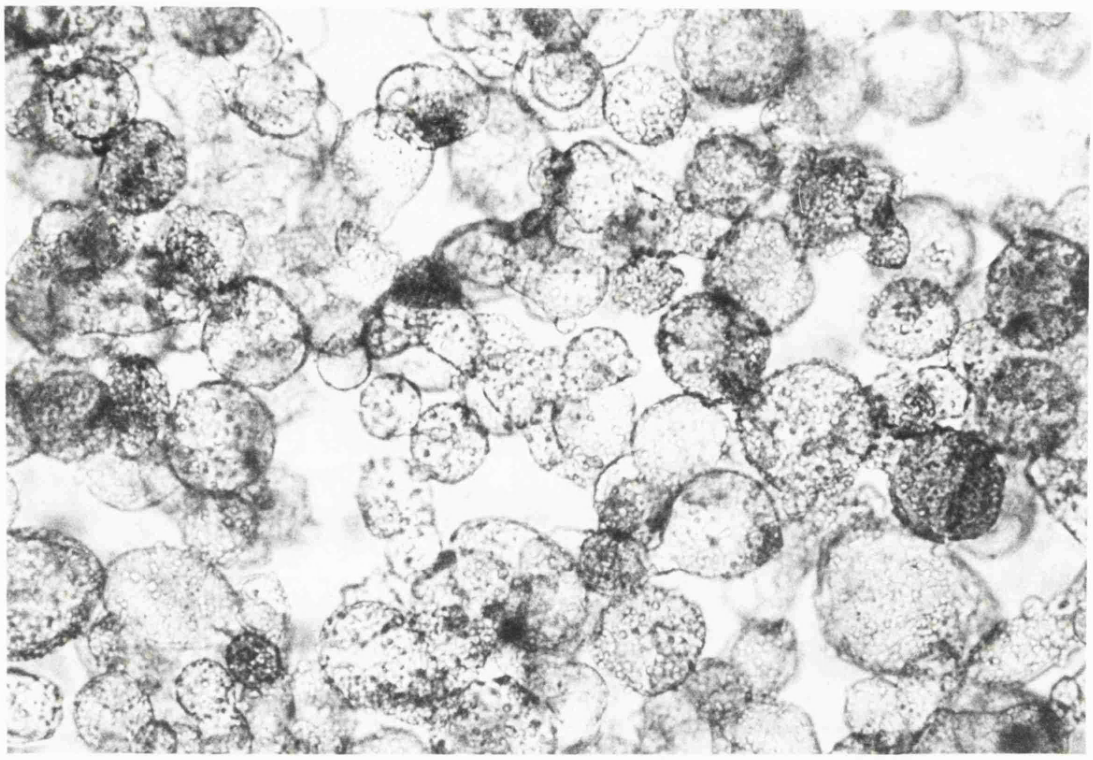
Preparation: Polyphthalamide microcapsules containing gelatin were prepared as given for polyphthalamide microcapsules in Section 3.2 with the following exceptions: 'Solution 2' consisted of 17% w/v gelatin in distilled water, polyethyleneimine was not included in the formulation, the buffer concentration in 'Solution 1' was 0.45M and the Span 85 concentration was 0.53% v/v. 'Solution 2' was also warmed to reduce its viscosity so that it could be mixed with an equal volume of 'Solution 1'.

Polyphthalamide microcapsules containing crosslinked gelatin were prepared as above. After the addition of 75ml of 'Solution 4' stirring was continued for 1 hour and the flask placed in ice. 5ml formaldehyde solution (38% v/v) was added and the stirring continued at a reduced speed for 12 hours. The suspension was centrifuged, the supernatant discarded and the microcapsules washed with 50ml cyclohexane.

Properties: Figure 3.17 shows a photograph of polyphthalamide microcapsules containing gelatin. In contrast to simple polyphthalamide microcapsules the interiors are not clear and the microcapsules are larger. Crosslinked gelatin containing microcapsules had a similar appearance. The size distributions of two batches of gelatin containing microcapsules are given in Figure 3.18 and the median volume diameters of the two batches are approximately 32μm and 34μm. Figure 3.19 and Table 3.23 show the release of pilocarpine nitrate from polyphthalamide microcapsules containing gelatin and crosslinked gelatin. It is apparent that the time taken for 90% of the total pilocarpine nitrate recovered to be released from the microcapsules was approximately 1 minute and 3 minutes for crosslinked gelatin and gelatin containing microcapsules respectively.

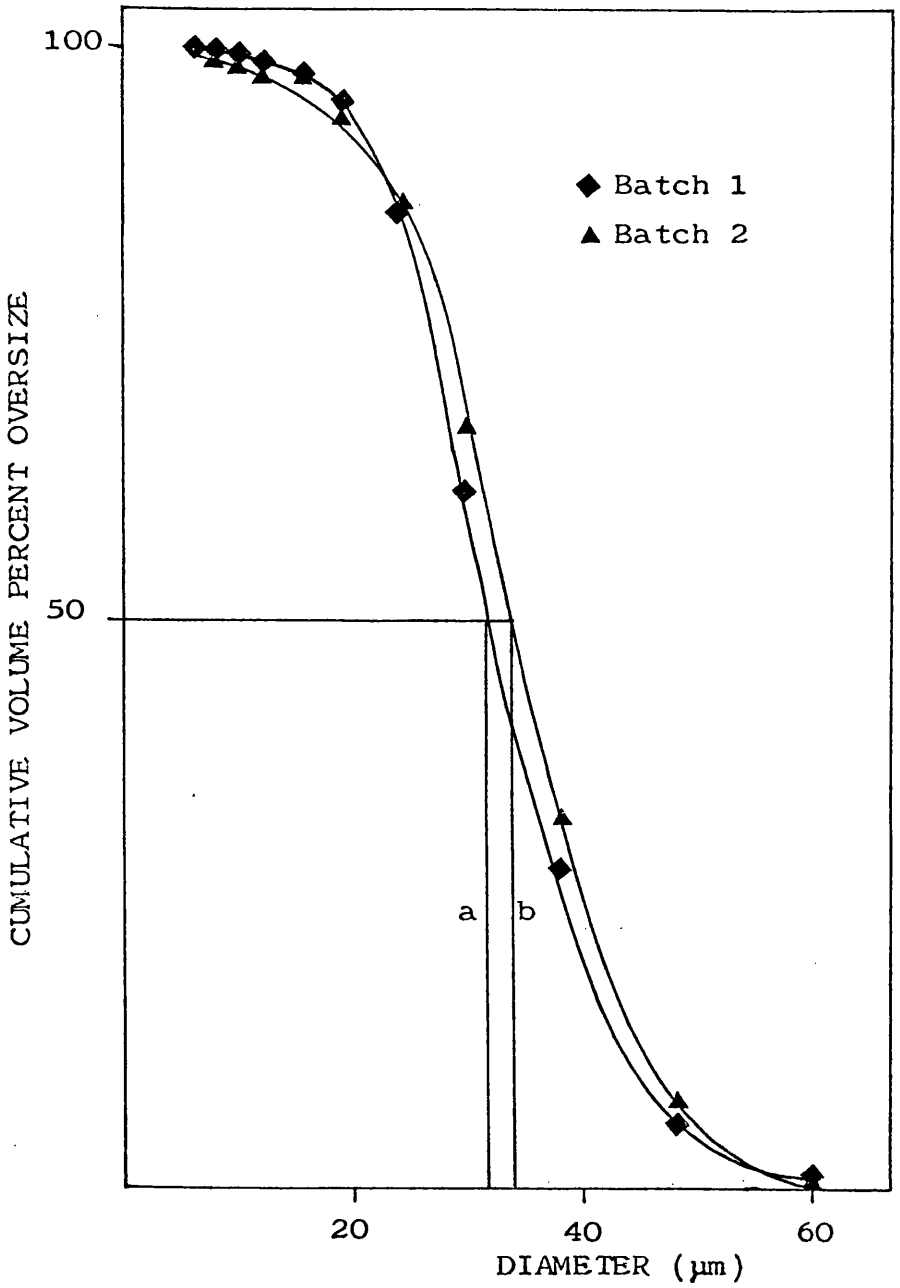
3.6.2 Polyphthalamide Microcapsules containing Bovine Serum Albumin

Preparation: Polyphthalamide microcapsules containing bovine serum albumin were prepared as given for polyphthalamide microcapsules (see Section 3.2) with the following exceptions. The concentration of polyethyleneimine in 'Solution 1' was 10% w/v, 'Solution 2' consisted of 5% w/v bovine serum albumin in distilled water and the Span 85 concentration was 0.13% v/v.



Scale: 50μm

FIGURE 3.17 POLYPHTHALAMIDE MICROCAPSULES CONTAINING GELATIN DISPERSED IN WATER.



a. Batch 1, median volume diameter 32μm
b. Batch 2, median volume diameter 34μm

FIGURE 3.18 SIZE DISTRIBUTION OF TWO BATCHES OF POLYPHTHALAMIDE MICROCAPSULES CONTAINING GELATIN.

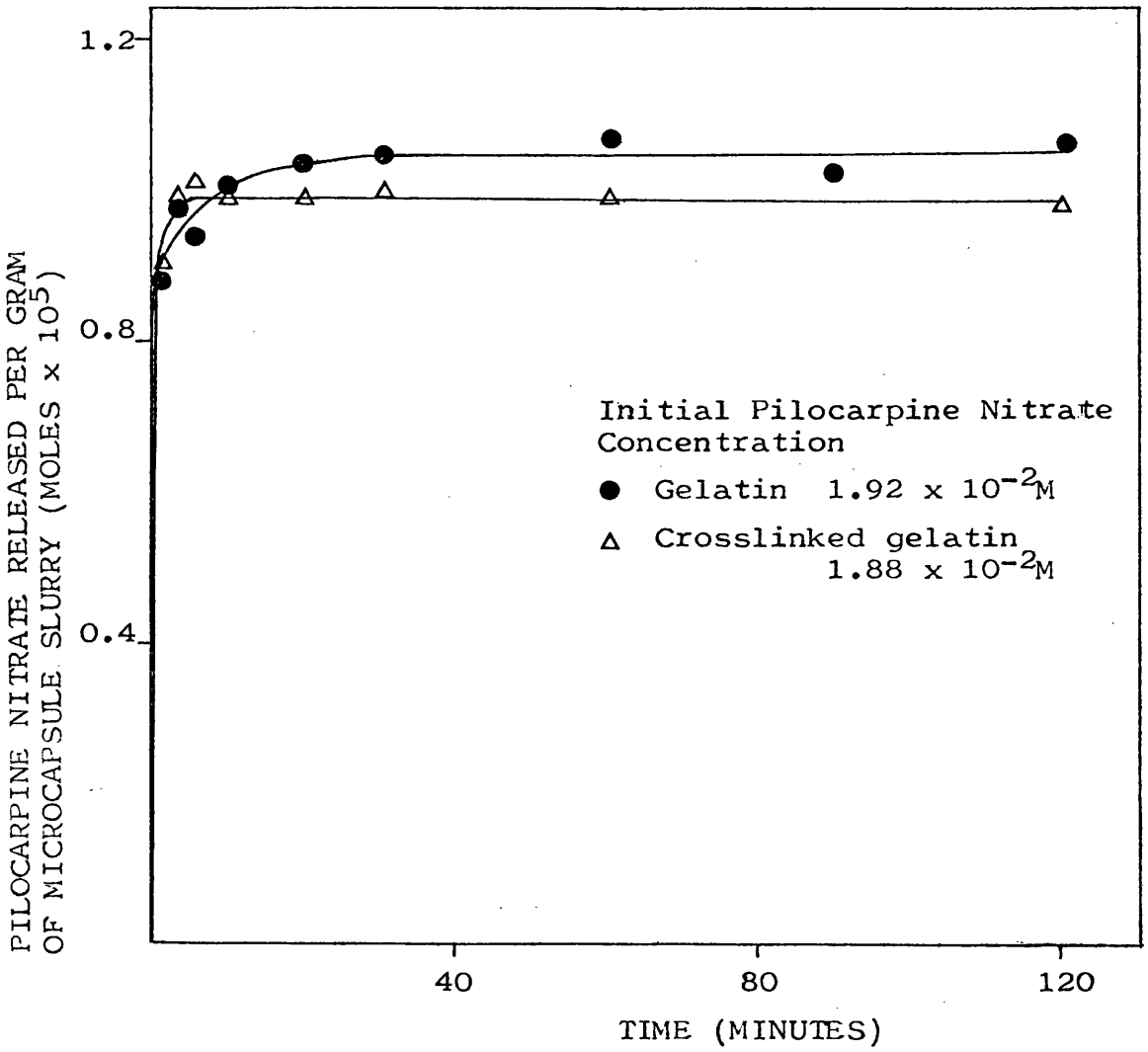


FIGURE 3.19 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES CONTAINING GELATIN AND CROSSLINKED GELATIN INTO pH 7.4 BUFFER AT 32°C.

	Gelatin.		Crosslinked Gelatin.	
Initial Pilocarpine Nitrate Concentration	1.92×10^{-2}M		1.88×10^{-2}M	
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.44×10^{-4} moles		1.41×10^{-4} moles	
Sample Weight taken from Microcapsule Slurry.	5.006g		6.494g	
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals.	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes. %	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes. %
Time (minutes) 1	0.879	81.9	0.909	93.1
3	0.978	91.1	0.989	101.3
5	0.940	87.6	1.009	103.4
10	1.013	94.4	0.989	101.3
20	1.038	96.7	0.987	101.1
30	1.049	97.8	1.000	102.5
60	1.074	100.1	0.988	101.2
90	1.029	95.9	-	-
120	1.073	100.0	0.976	100.0
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.373		0.471	

TABLE 3.23 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES CONTAINING GELATIN AND CROSSLINKED GELATIN INTO pH 7.4 BUFFER AT 32°C.

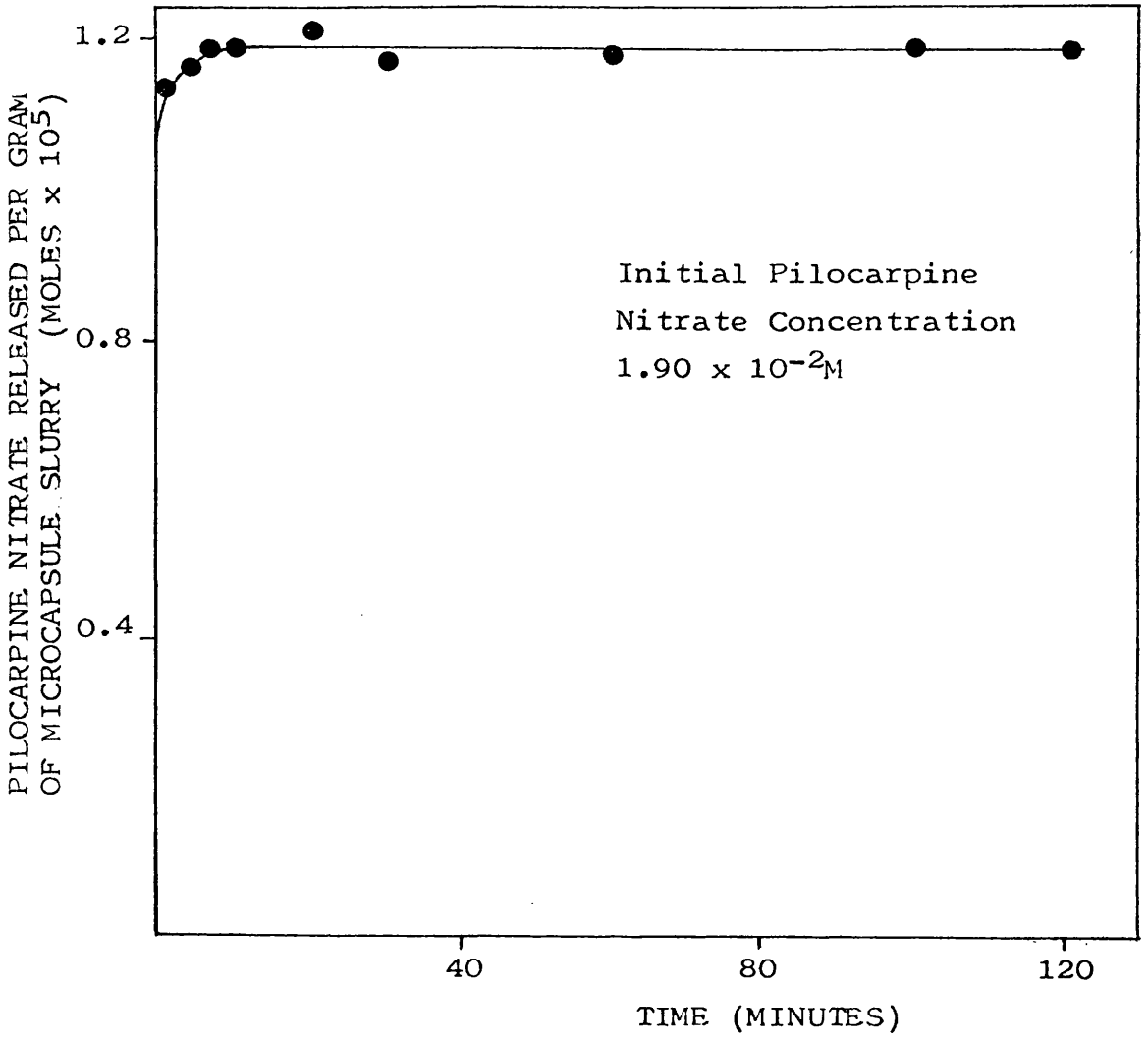


FIGURE 3.20 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES CONTAINING BOVINE SERUM ALBUMIN INTO pH 7.4 BUFFER AT 32°C.

Initial Pilocarpine Nitrate Concentration.	1.90×10^{-2}M	
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.427×10^{-4}moles	
Sample Weight taken from Microcapsule Slurry.	4.8548g	
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals.	Moles Released per gram of Microcapsule Slurry. $\times 10^{5}$	Percent of Total Mass Released in 120 Minutes.
Time (minutes)		
1	1.136	95.8
4	1.172	98.8
6	1.185	99.9
10	1.187	100.1
20	1.211	102.1
30	1.170	98.7
60	1.177	99.3
90	1.189	100.2
120	1.186	100.0
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.403	

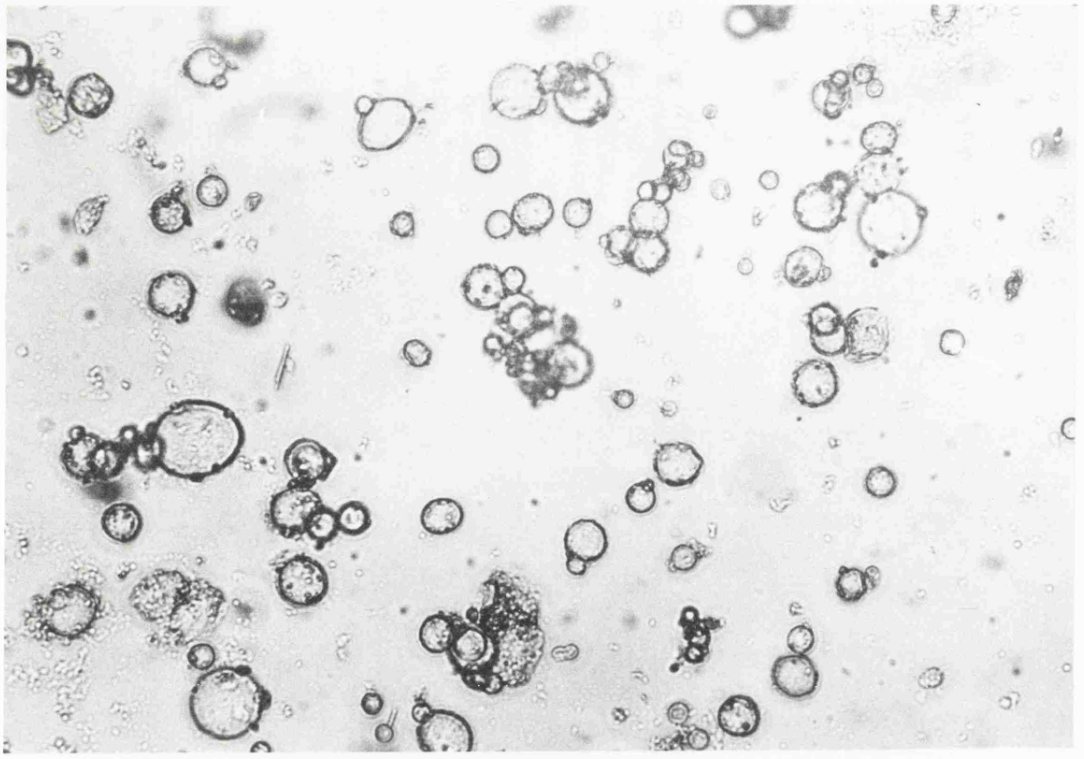
TABLE 3.24 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES CONTAINING BOVINE SERUM ALBUMIN INTO pH 7.4 BUFFER AT 32°C.

Release of Pilocarpine Nitrate: Figure 3.20 and Table 3.24 show the release of pilocarpine nitrate from bovine serum albumin containing microcapsules which was found to be rapid. Approximately 95% of the total amount of pilocarpine nitrate released was lost in the first minute.

3.6.3 Polyphthalamide Microcapsules prepared using Short Chain Crosslinking Molecules

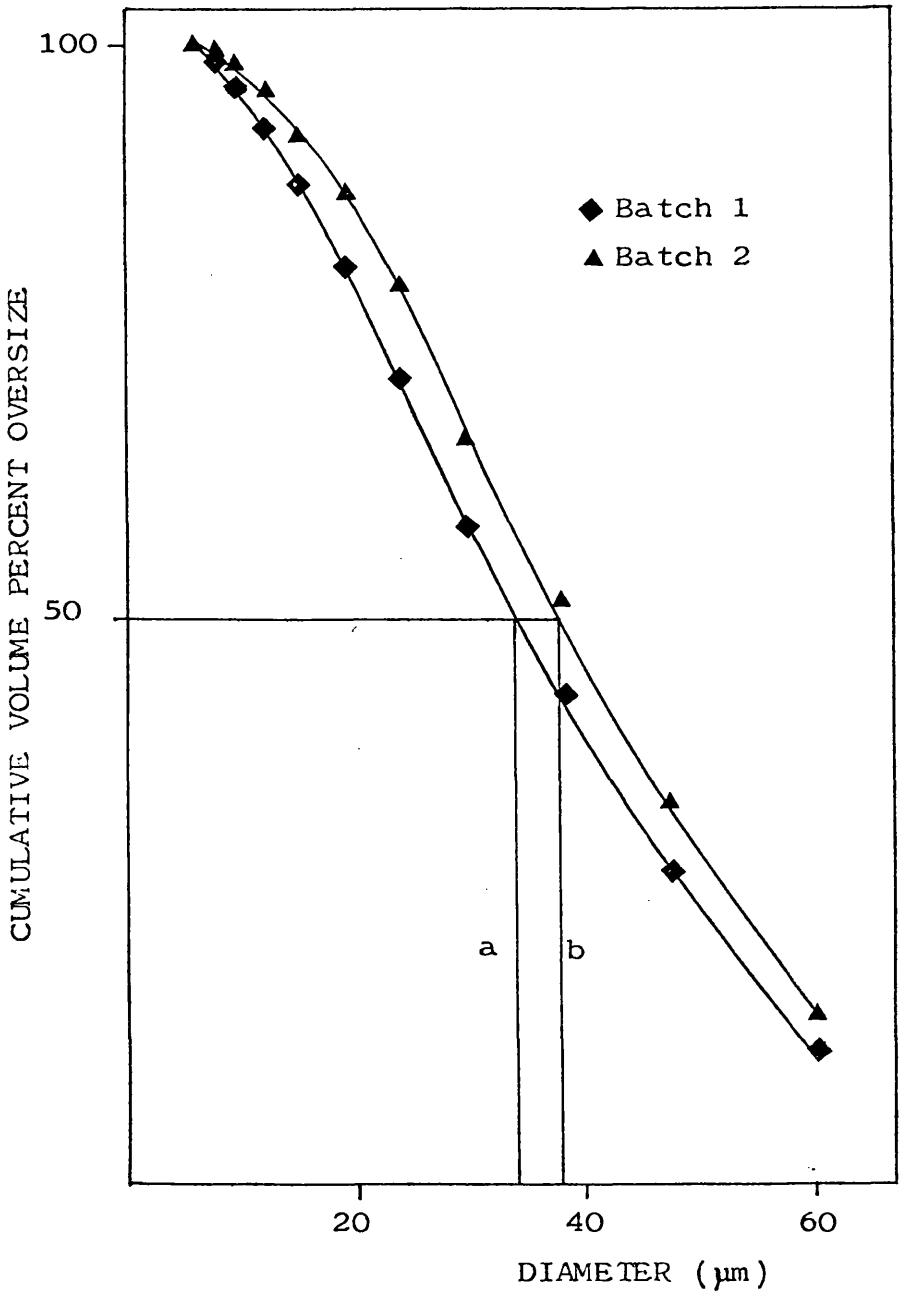
Preparation: Polyphthalamide microcapsules with crosslinked walls prepared using the short chain crosslinking molecules diethylenetriamine and 1,3,5 benzentricarboxylic acid chloride were prepared as follows. 'Solutions 1' and '3' were the same as described for polyphthalamide microcapsule preparation (see Section 3.2) except that the Span 85 concentration was 0.13% v/v, and polyethyleneimine was not included. The buffer concentration was 0.45M. 'Solution 2' consisted of 0.4M diethylenetriamine in distilled water and 'Solution 4' was composed of 0.044M phthaloyl chloride and 0.044M 1,3,5 benzenetricarboxylic acid chloride in chloroform: cyclohexane 1 part to 5 parts by volume containing 0.13% Span 85. The method of preparation was as for polyphthalamide microcapsules except that after the addition of 'Solution 4' the suspension was stirred for 30 minutes before pouring into cyclohexane.

Properties: Figure 3.21 shows the appearance of polyphthalamide microcapsules prepared using short chain crosslinking molecules. As can be seen the majority of the microcapsules are spherical but there are a large proportion of aggregates present. The size distributions of two typical batches of these microcapsules are illustrated in Figure 3.22. The median volume diameters



Scale: 50μm

FIGURE 3.21 POLYPHTHALAMIDE MICROCAPSULES PREPARED USING SHORT CHAIN CROSSLINKING MOLECULES DISPERSED IN WATER.



a. Batch 1, median volume diameter 34μm
b. Batch 2, median volume diameter 38μm

FIGURE 3.22 SIZE DISTRIBUTION OF TWO BATCHES OF POLYPHTHALAMIDE MICROCAPSULES PREPARED USING SHORT CHAIN CROSSLINKING MOLECULES.

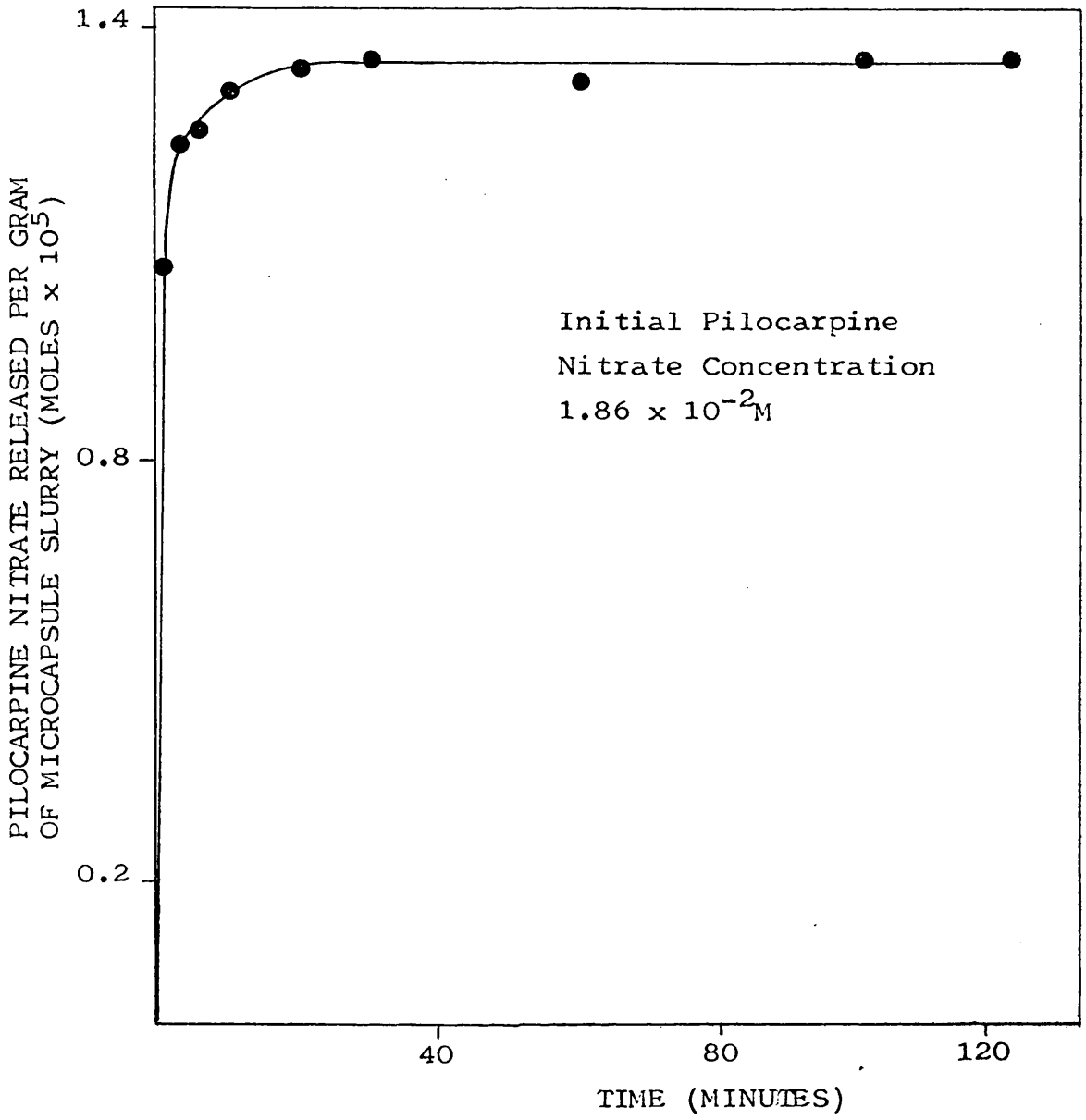


FIGURE 3.23 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES PREPARED USING SHORT CHAIN CROSSLINKING MOLECULES INTO pH 7.4 BUFFER AT 32°C.

Initial Pilocarpine Nitrate Concentration.	1.86×10^{-2}M	
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.395×10^{-4}moles	
Sample Weight taken from Microcapsule Slurry.	5.9640g	
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals.	Moles Released per gram of Microcapsule Slurry. $\times 10^5$	Percent of Total Mass Released in 120 Minutes.
Time (minutes)		
1	1.076	78.8
3	1.246	91.2
5	1.262	92.4
10	1.323	96.8
20	1.354	99.1
30	1.363	99.8
60	1.331	97.4
90	1.363	99.8
120	1.366	100.0
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.584	

TABLE 3.25 THE RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES PREPARED USING SHORT CHAIN CROSSLINKING MOLECULES INTO pH 7.4 BUFFER AT 32°C.

of the two batches are 34μm and 38μm and there is a relatively high percentage of particles with diameters in the 40μm to 60μm size range. Figure 3.23 and Table 3.25 show the release of pilocarpine nitrate from the microcapsules. The release was rapid, approximately 90% of the total drug recovered being released in the first five minutes.

3.6.4 Polyphthalamide Microcapsules prepared by a Double Polymerisation Technique

Preparation: Polyphthalamide microcapsules were prepared by a double polymerisation technique using two methods. These are referred to as double walled microcapsules.

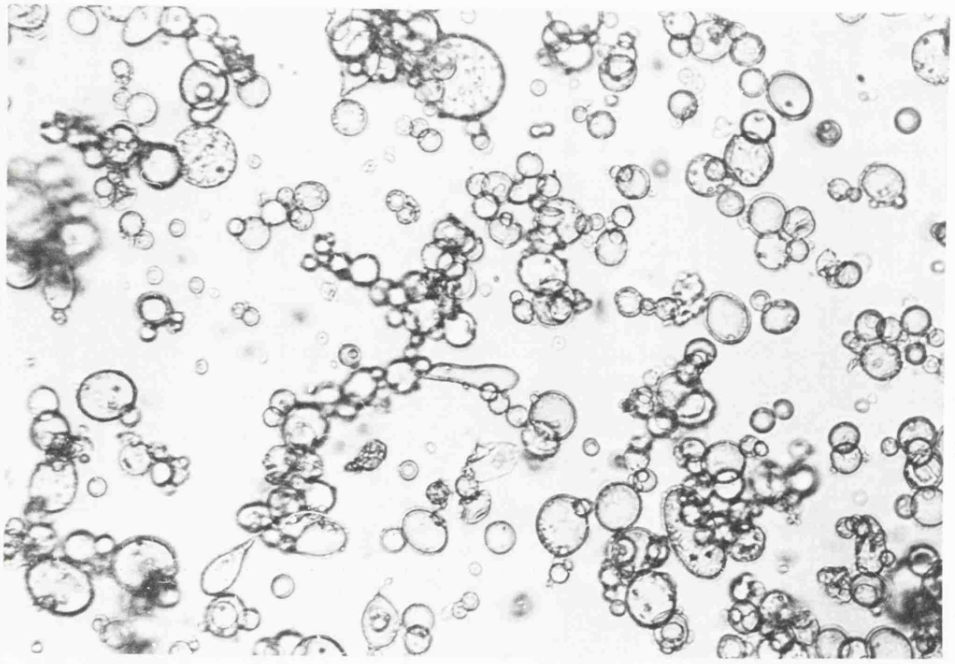
Double Walled Microcapsules Method A: Microcapsules were prepared as described for polyphthalamide microcapsules in Section 3.2. After the addition of 75ml 'Solution 4' and stirring for 3 minutes the suspension was poured into 100ml cyclohexane and centrifuged. The supernatant was removed and the microcapsules resuspended in 75ml 1% Span 85 in cyclohexane. Whilst stirring at 2,000 rpm 10ml of a solution consisting of 0.33M phthaloyl chloride in chloroform:cyclohexane 1 part to 5 parts by volume containing 1% v/v Span 85 was added. Stirring was continued for 3 minutes after which the suspension was centrifuged, the supernatant discarded and the microcapsules washed using 50ml cyclohexane.

Double Walled Microcapsules Method B: Microcapsules were prepared as described for polyphthalamide microcapsules in Section 3.2. After the addition of 75ml 'Solution 4' and stirring for 3 minutes the suspension was poured into 50ml cyclohexane and centrifuged. The microcapsules were harvested, freeze dried for 5 hours and immersed in 6ml equivolume aqueous 0.4M piperazine solution and 0.45M carbonate-bicarbonate buffer containing 10% w/v polyethyleneimine. Following centrifugation the supernatant was removed, the microcapsules were resuspended in 75ml 'Solution 3' and the suspension was stirred at 2,000 rpm in a 250ml conical flask for 1 minute. 75ml 'Solution 4' was added and stirring continued for 3 minutes. The suspension was poured into 100ml cyclohexane and the microcapsules again centrifuged. The supernatant was removed and the microcapsules dispersed in 50ml cyclohexane. This suspension was then centrifuged and the supernatant discarded.

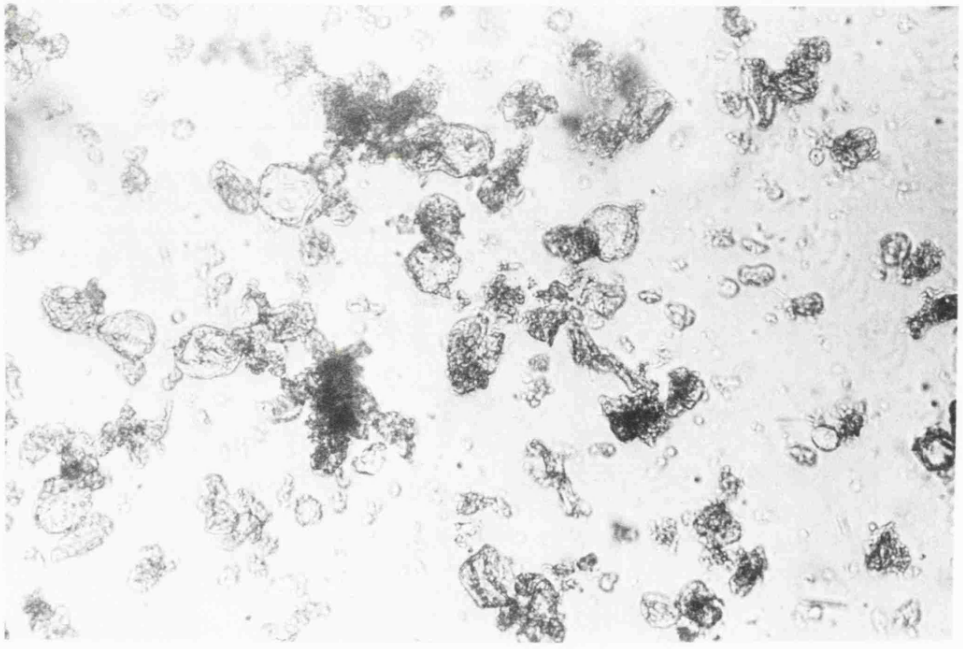
Properties: Figure 3.24 shows the appearance of double walled polyphthalamide microcapsules. The interiors are clear and the majority of type A microcapsules are spherical. A large number of aggregates are present in the microcapsules prepared using Method B. There are also a large proportion of what appear to be small lumps of polymer present and the microcapsules are generally crenated. The size distributions of the two types of microcapsule are given in Figure 3.25 which shows that in both cases the median volume diameter is approximately 25μm. The double walled microcapsule suspensions prepared by Method B however consist of a larger proportion of particles in the 40μm to 60μm size range. The release

of pilocarpine nitrate from the two types of double walled microcapsules is given in Figure 3.26 and Table 3.26. It may be seen from Figure 3.26 that the pilocarpine nitrate was released rapidly. In the case of the microcapsules prepared using Method A 90% of the mass of pilocarpine nitrate released in 120 minutes was released within the first minute. A similar value was obtained for microcapsules prepared using Method B although from the release profile it appears that the remaining pilocarpine nitrate was released more slowly.

a.



b.



Scale: 50μm

FIGURE 3.24 DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED USING a) METHOD A AND b) METHOD B AFTER DISPERSION IN WATER.

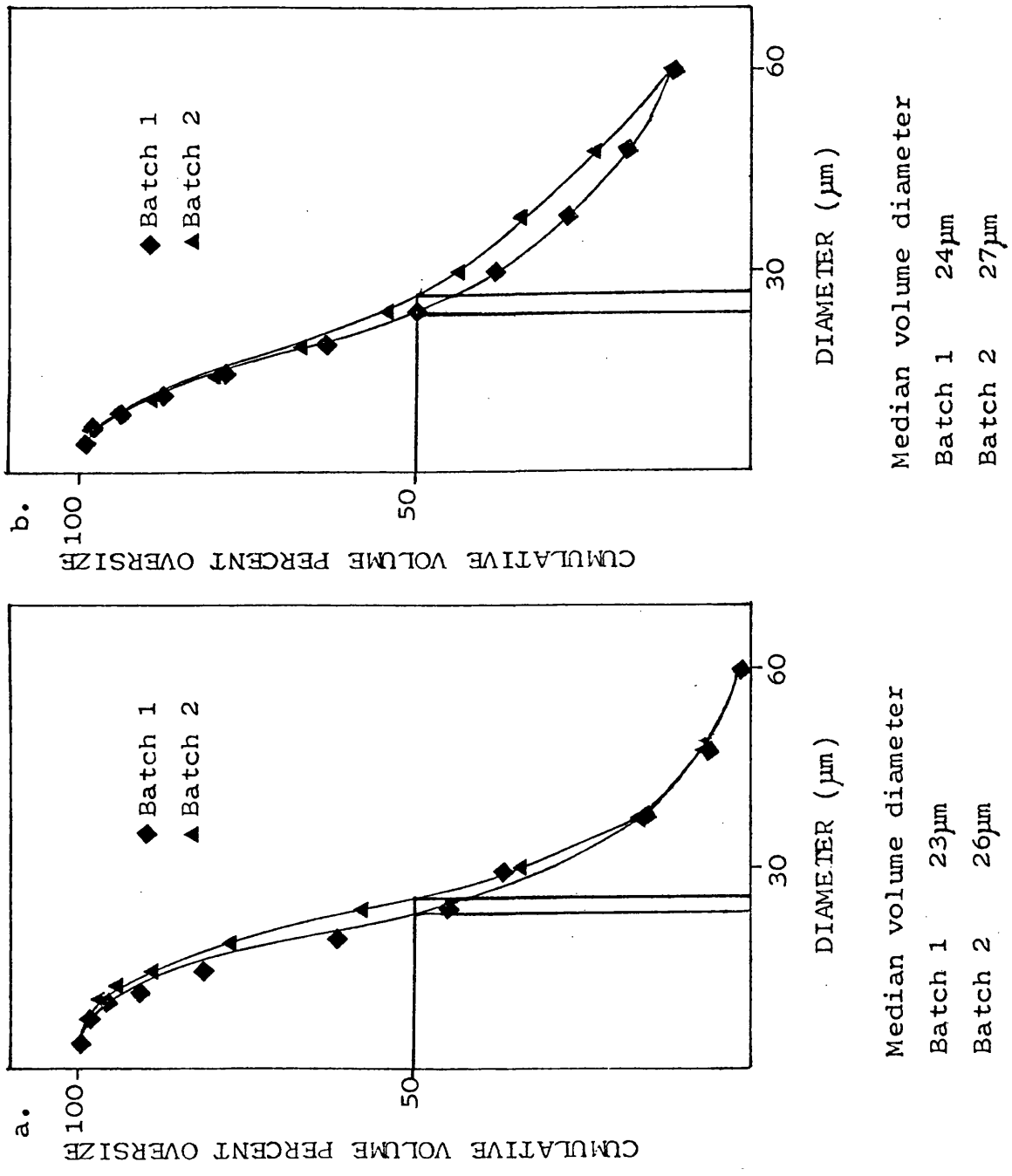


FIGURE 3.25 SIZE DISTRIBUTION OF TWO BATCHES OF DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED USING a) METHOD A AND b) METHOD B.

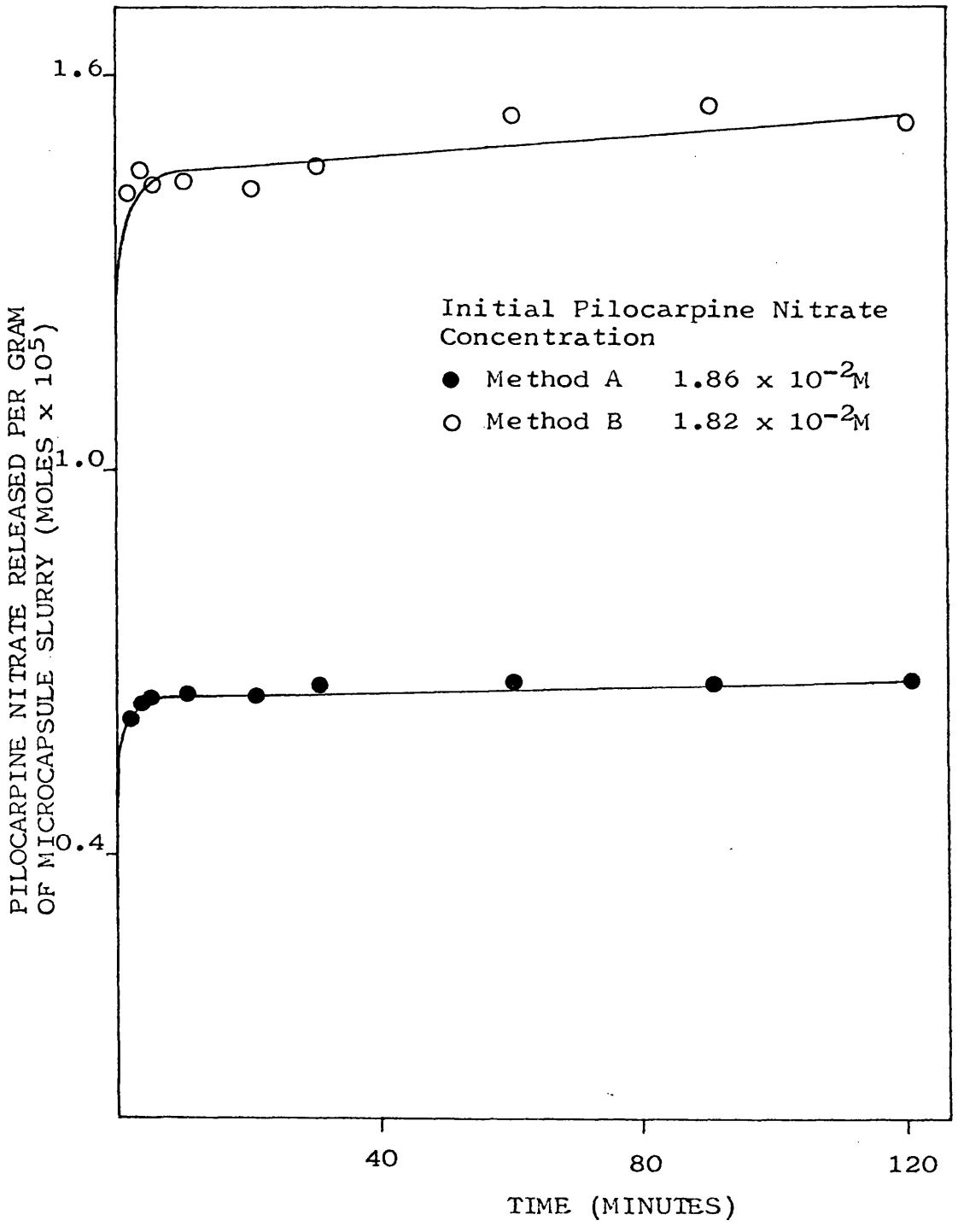


FIGURE 3.26 THE RELEASE OF PILOCARPINE NITRATE FROM DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED BY METHOD A AND METHOD B INTO pH 7.4 BUFFER AT 32°C.

	Double Walled Method A		Double Walled Method B	
Initial Pilocarpine Nitrate Concentration.	1.86×10^{-2}M		1.82×10^{-2}M	
Mass of Pilocarpine Nitrate in Initial Aqueous Phase.	1.396×10^{-4} moles		1.367×10^{-4} moles	
Sample Weight taken from Microcapsule Slurry.	5.6773g		1.7597g	
Release of Pilocarpine Nitrate from Microcapsules at Timed Intervals.	Moles Released per gram of Microcapsule Slurry. x 10^5	Percent of Total Mass Released in 120 minutes. %	Moles Released per gram of Microcapsule Slurry x 10^5	Percent of Total Mass Released in 120 minutes. %
Time (minutes) 1	0.616	92.1	1.419	93.5
3	0.624	93.3	1.452	95.7
5	0.642	96.0	1.433	94.4
10	0.644	96.3	1.435	94.5
20	0.645	96.4	1.424	93.8
30	0.658	98.4	1.459	96.1
60	0.673	100.5	1.548	102.0
90	0.666	99.6	1.550	102.1
120	0.669	100.0	1.518	100.0
24 hours	0.683	102.1	1.612	106.2
Mass of Pilocarpine Nitrate Released Expressed as Fraction of Total Mass Included.	0.277		0.208	

TABLE 3.26 THE RELEASE OF PILOCARPINE NITRATE FROM DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED BY METHOD A AND METHOD B INTO pH 7.4 BUFFER AT 32°C.

3.7 Electron Microscopy of Microcapsule Walls

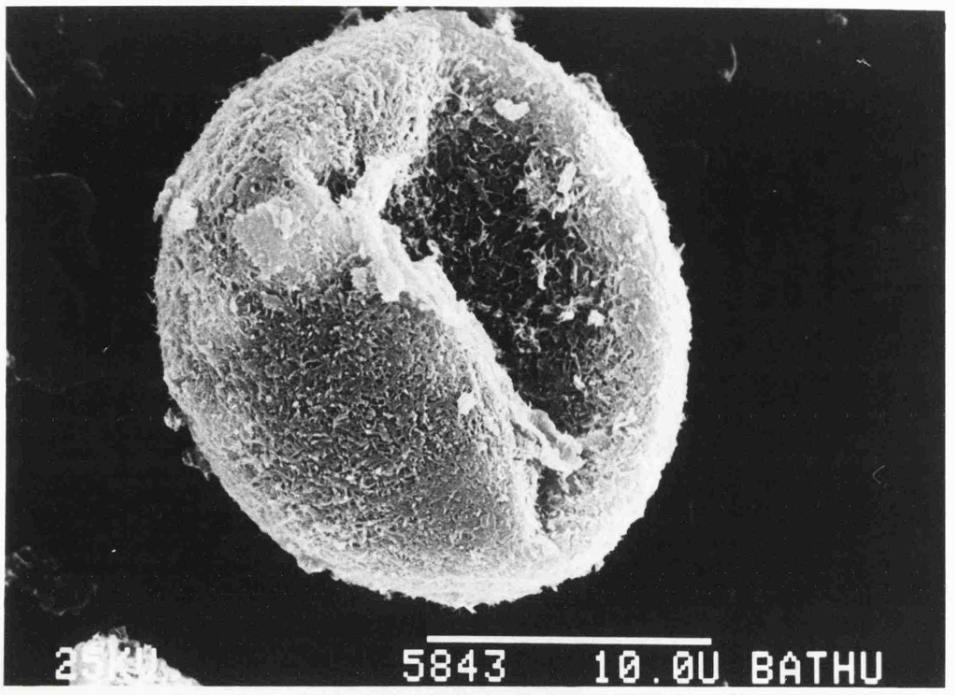
Detail of the microcapsule walls was investigated using electron microscopy. The method used for scanning electron microscopy of samples prepared by a critical point drying technique is outlined in Section 3.3.1. For transmission electron microscopy microcapsule suspensions were prepared in acetone. The samples together with an aqueous solution of ruthenium red were placed in a plastic vial and rotated. Rotation was then continued for 12 hours after which the acetone water mixture was replaced by an epoxy resin. The resin was hardened by heating in an oven for 12 hours and sectioned using a microtome. Following sectioning, the layers were mounted on copper grids and stained with lead citrate and uranyl acetate.

Scanning electron micrographs of the surface of a polyphthalamide microcapsule are shown in Figure 3.27. The microcapsule surface is seen to be extremely uneven with large indentations, possibly pores or voids of approximately 0.1μm diameter. Figure 3.28 shows transmission electron micrographs of sectioned polyphthalamide microcapsule walls illustrating the appearance of a transverse section of the walls. The wall is estimated to be 0.6 to 0.8μ in thickness and there are three clearly distinct regions within it.

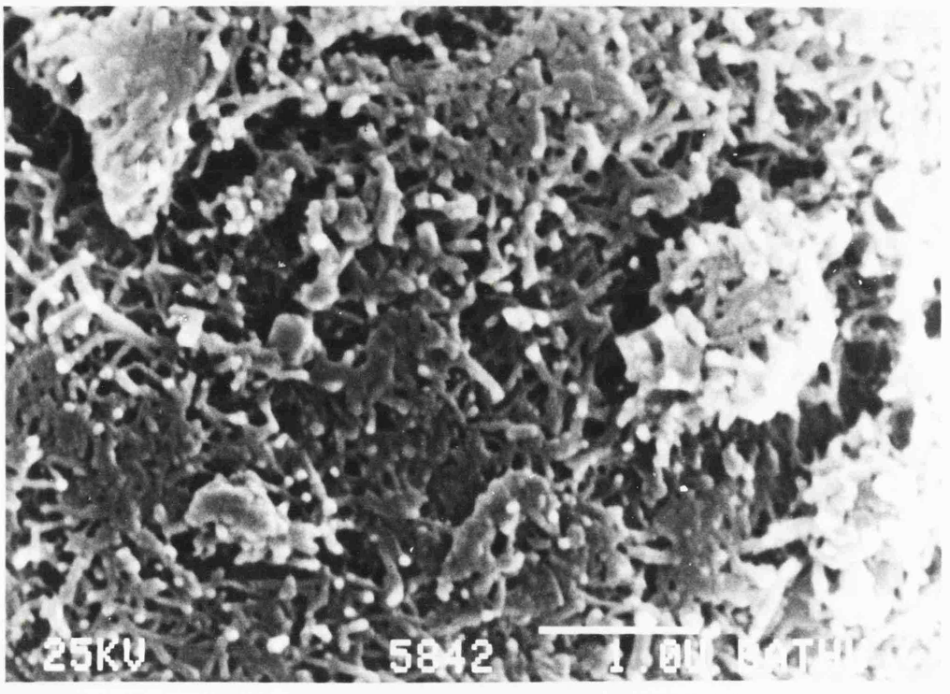
From the core outwards:

1. a dense discontinuous nodular layer
2. a narrow dense continuous layer and
3. a relatively thick loosely attached, less dense layer

To ensure that the nodular layer was part of the wall and not due to the resin or the ruthenium red its composition was analysed using the EDAX (energy dispersive X-ray analysis) facility attached

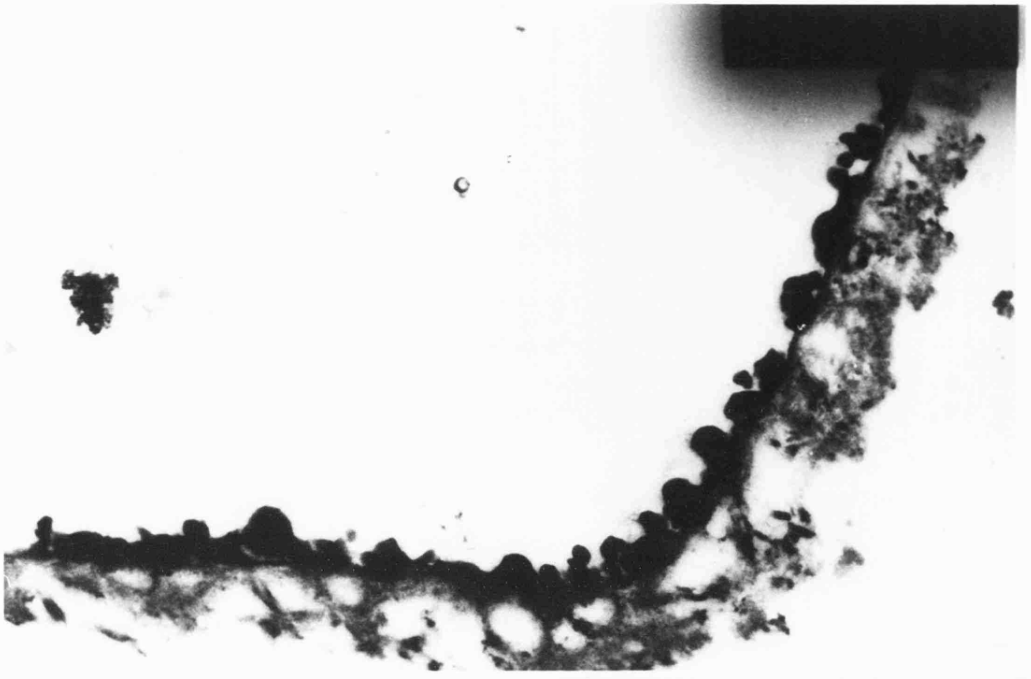


Scale: ⊢——⊣
10.0μm

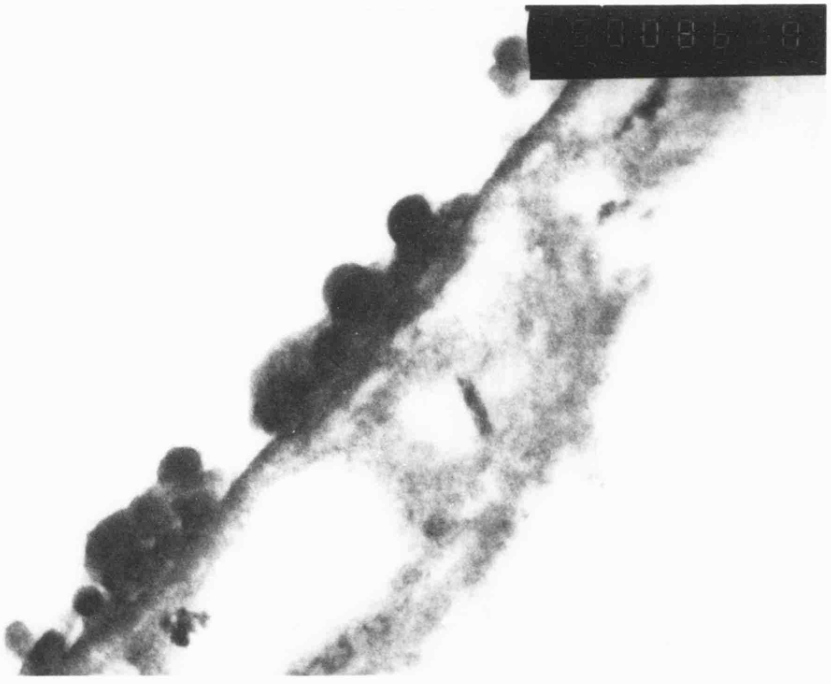


Scale: ⊢——⊣
1.0μm

FIGURE 3.27 SCANNING ELECTRON MICROGRAPHS OF THE SURFACE OF A POLYPHTHALAMIDE MICROCAPSULE.



Magnification: x 24000 Scale: 1.0μm



Magnification: x 75000 Scale: 0.2μm

FIGURE 3.28 TRANSMISSION ELECTRON MICROGRAPHS OF A SECTION OF THE WALL OF A POLYPHTHALAMIDE MICROCAPSULE.

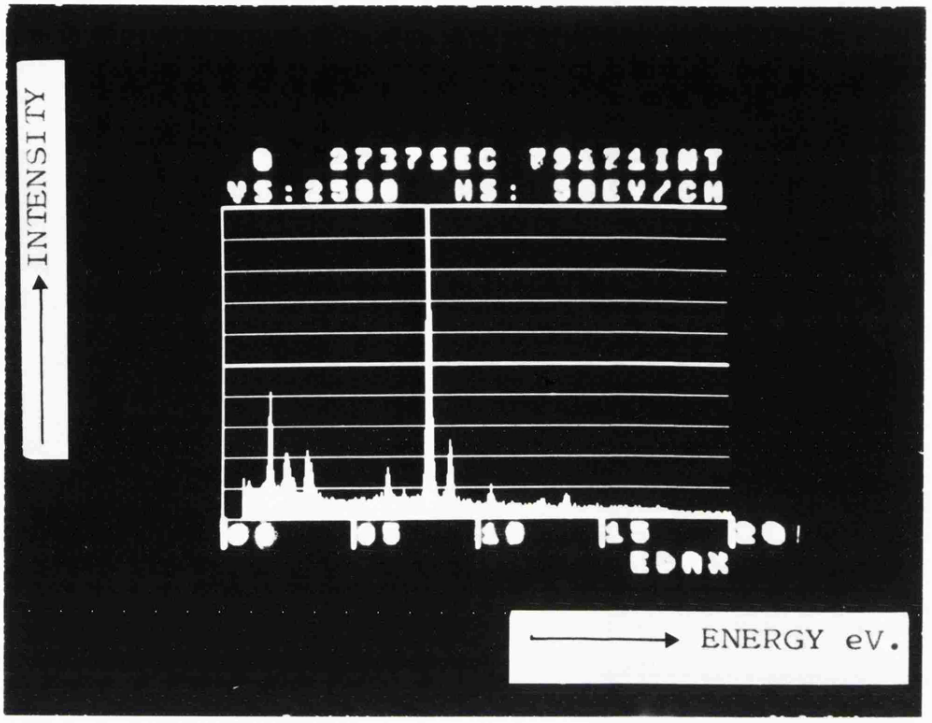
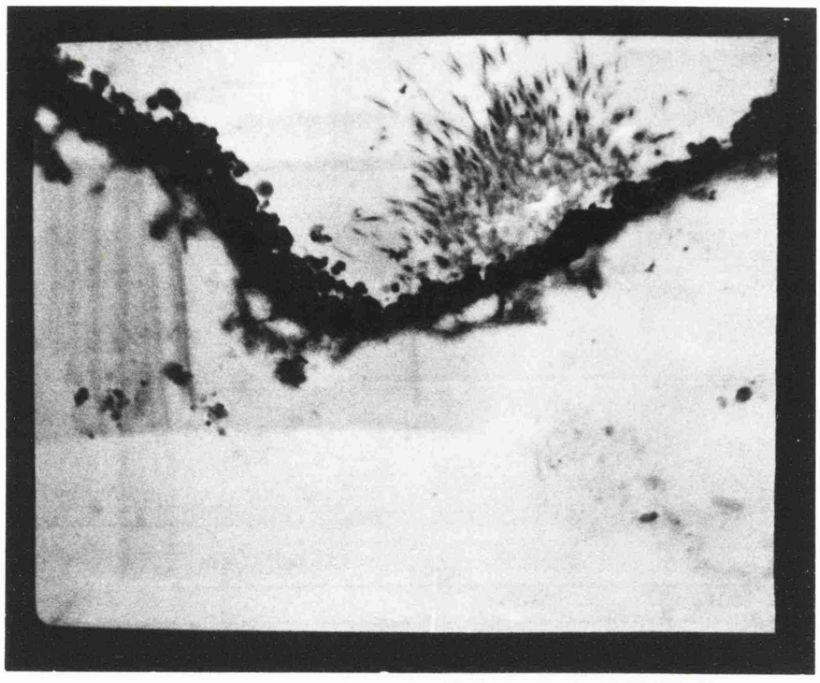


FIGURE 3.29 EDAX ANALYSIS OF THE NODULAR LAYER OF A POLYPHTHALAMIDE MICROCAPSULE WALL.

a.



b.

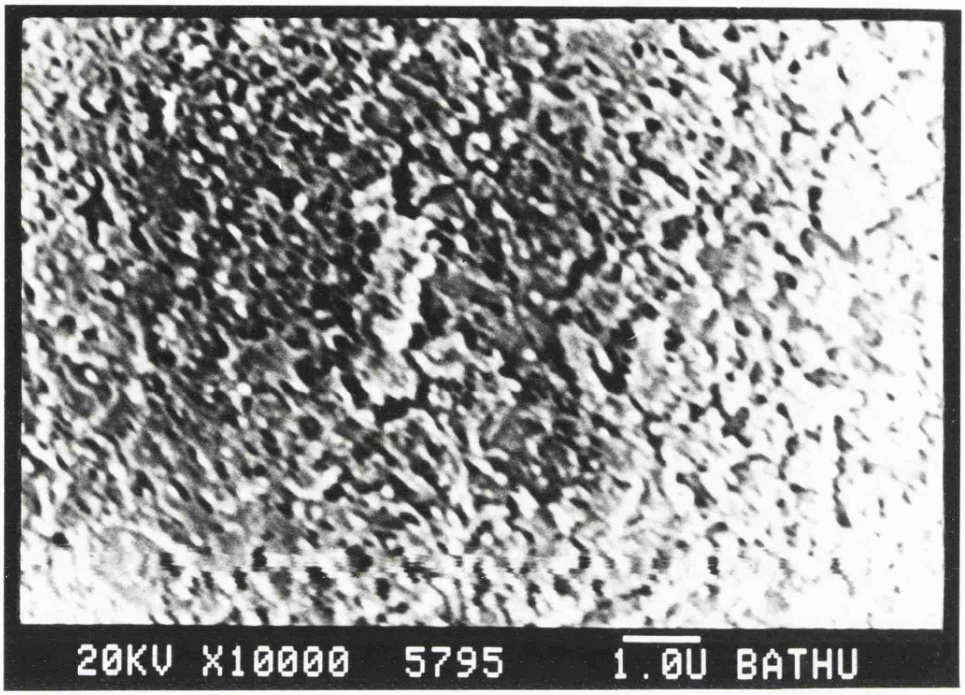
FIGURE 3.30 EDAX ANALYSIS OF A POLYPHTHALAMIDE MICROCAPSULE WALL.

a) Image of microcapsule wall
b) X-ray emissions from specimen corresponding to 3.2eV.

to the transmission electron microscope. This technique allows the distribution of certain elements within a sample to be identified and analysed semi-quantitatively. Analysis of a point in a nodule present in the section of wall shown in Figure 3.30a resulted in the spectrum given in Figure 3.29. The large peak at 8 eV corresponds to copper which was the material used to form the mounting. A peak is also seen at 3.2 eV which is the M peak for uranium. The entire specimen shown in Figure 3.30a was then scanned for emissions corresponding to 3.2 eV and the picture in Figure 3.30b constructed. The image of the microcapsule wall and the image of the emissions correpsonding to 3.2eV coincide indicating that the uranium acetate stain was present in the nodules and in the bulk of the wall. Since neither the resin nor the ruthenium red will stain with uranyl acetate (137) the observed inner layer must be part of the wall.

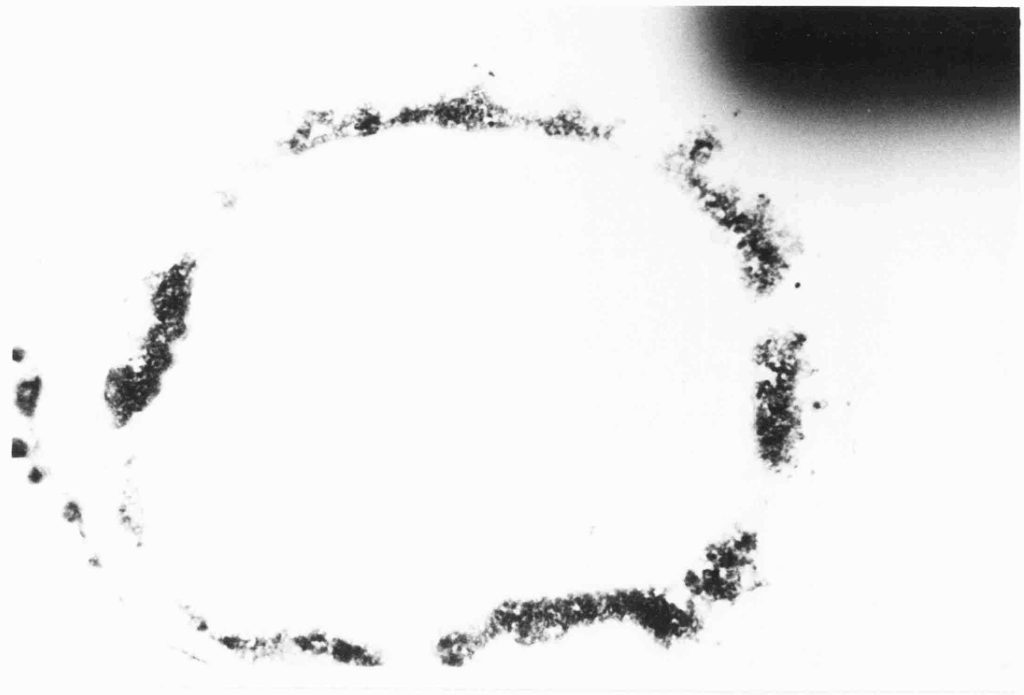
The appearance of the microcapsule walls prepared using the short chain crosslinking molecules is represented in Figure 3.31 from which no obvious difference between these microcapsule walls and those of polyphthalamide microcapsules is apparent. Again the surface is seen to be rough with discontinuities or pores present, the majority or which are less than 0.5μm diameter. The transmission electron micrographs (Figure 3.32) however do indicate a difference between the two types of capsule. The microcapsule section in Figure 3.32b which is estimated to be approximately 0.2 to 0.3μm in thickness consists of only two layers.

1. a dense discontinuous outer layer
2. a thin less dense continuous inner layer

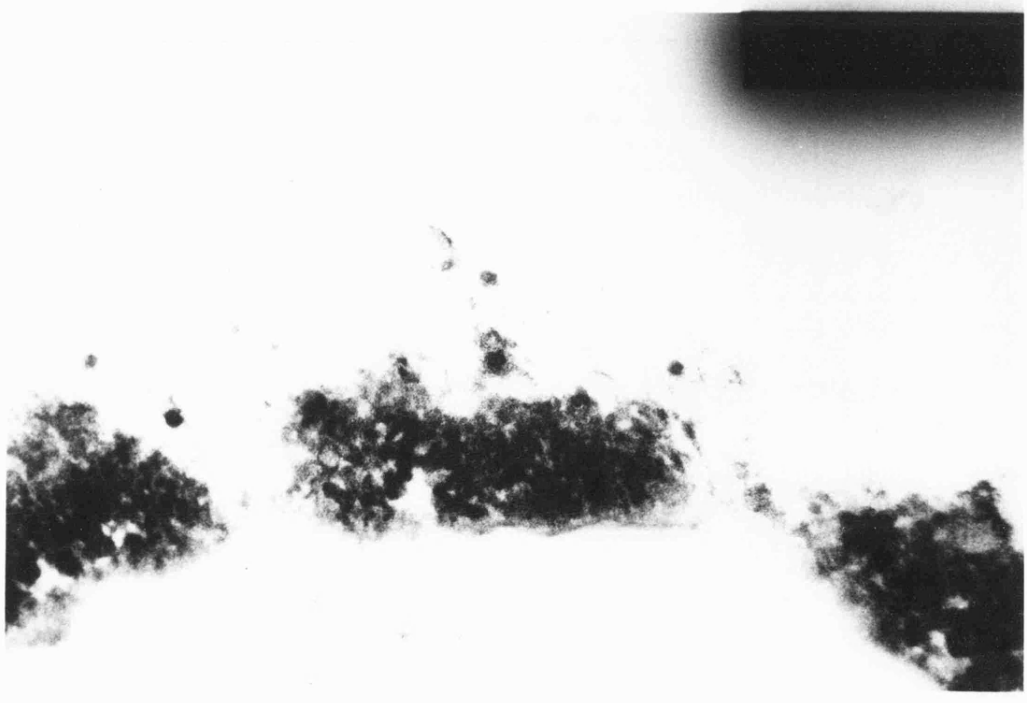


Scale: ⊢——⊣
1.0μm

FIGURE 3.31 SCANNING ELECTRON MICROGRAPH OF THE SURFACE OF POLYPHTHALAMIDE MICROCAPSULES PREPARED USING SHORT CHAIN CROSSLINKING MOLECULES.



Magnification: x 24000 Scale: 1.0μm

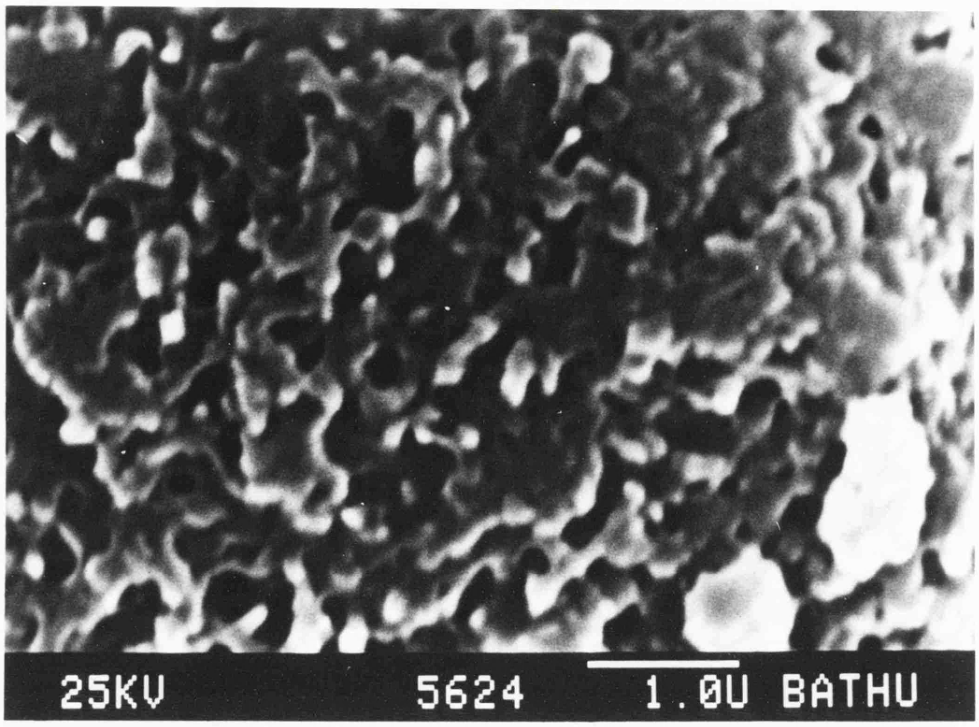


Magnification: x 75000 Scale: 0.2μm

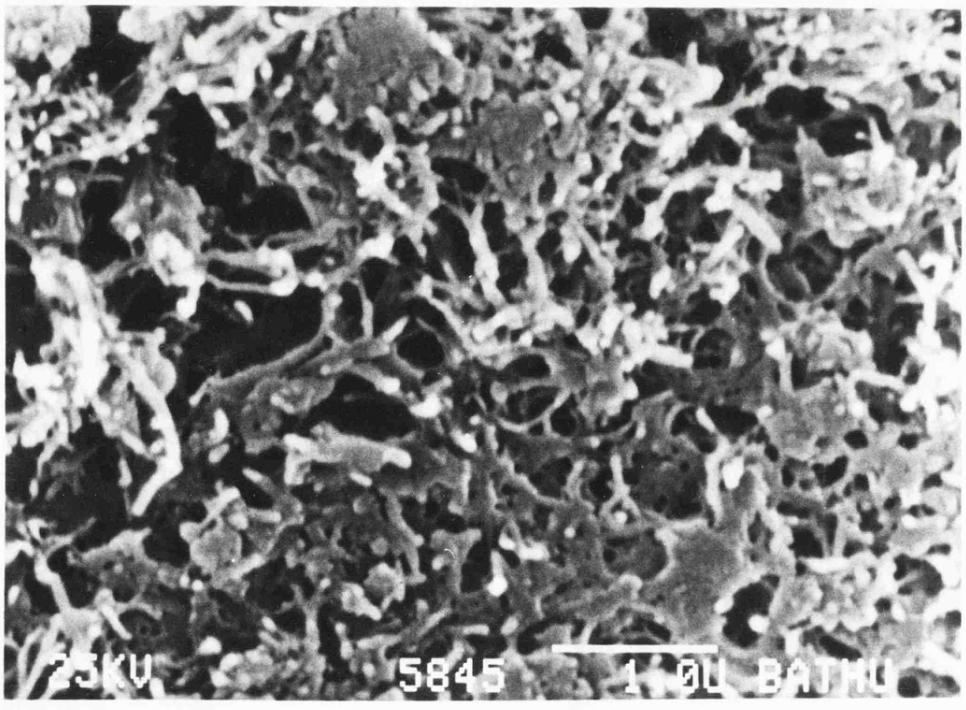
FIGURE 3.32 TRANSMISSION ELECTRON MICROGRAPHS OF THE WALL OF POLYPHTHALAMIDE MICROCAPSULES PREPARED USING SHORT CHAIN CROSSLINKING MOLECULES.

Figures 3.33, 3.34 and 3.35 are the scanning electron and transmission electron micrographs respectively of microcapsules prepared with double layer walls. The appearance of the outer surface of the walls (Figure 3.33) is the same as that for polyphthalamide microcapsules. The transmission electron micrographs however do show differences. The section from the microcapsules prepared by method A (Figure 3.34) consists of two regions, a nodular inner layer and a relatively dense outer layer. There is also some less dense material present on the surface. The wall thickness is estimated to be 0.4 to 0.5μm. The section from Type B double walled microcapsules clearly shows the presence of two distinct walls. Figure 3.35b shows the whole microcapsule from which the specimen in Figure 3.35a was taken. The microcapsule is collapsed to an extent where in parts the walls are touching and it is evident from Figure 3.35a that the wall is composed of two bands. The inner band is consistent with the structure found in polyphthalamide microcapsule walls. The outer band comprises an inner diffuse layer which in places appears hollow and an outer thin dense layer. This double wall is estimated to be approximately 0.3 to 0.4μm in thickness.

Gelatin microcapsules collapsed during the preparative technique for electron microscopy and therefore the structure of these microcapsules was not investigated using this method.

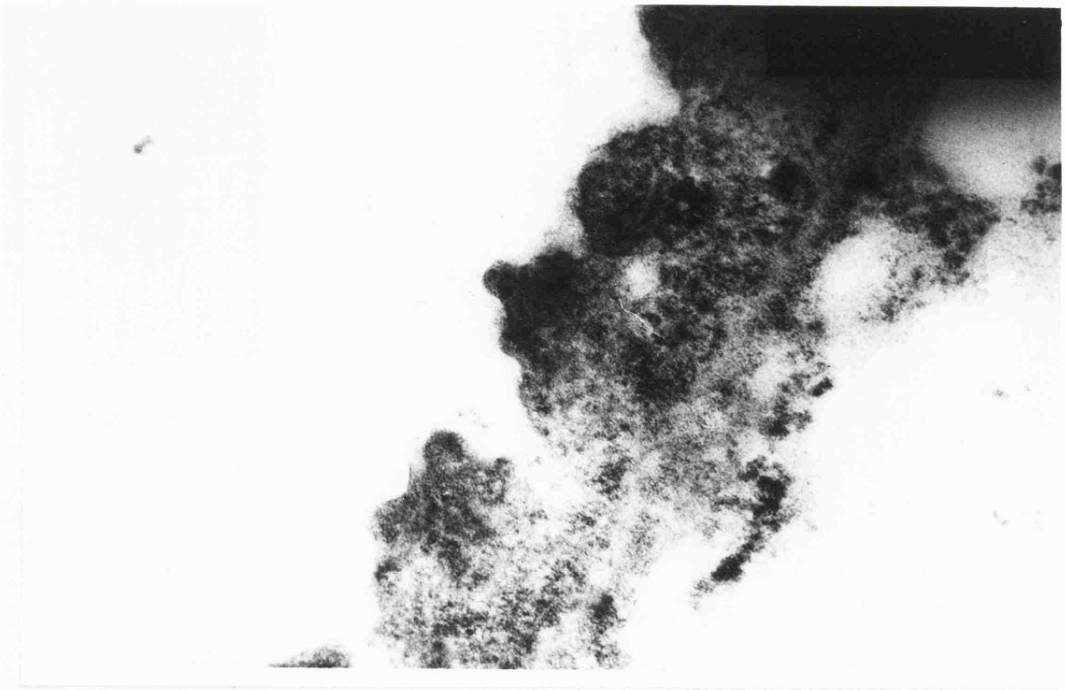


Scale: ⊢——⊣
1.0μm

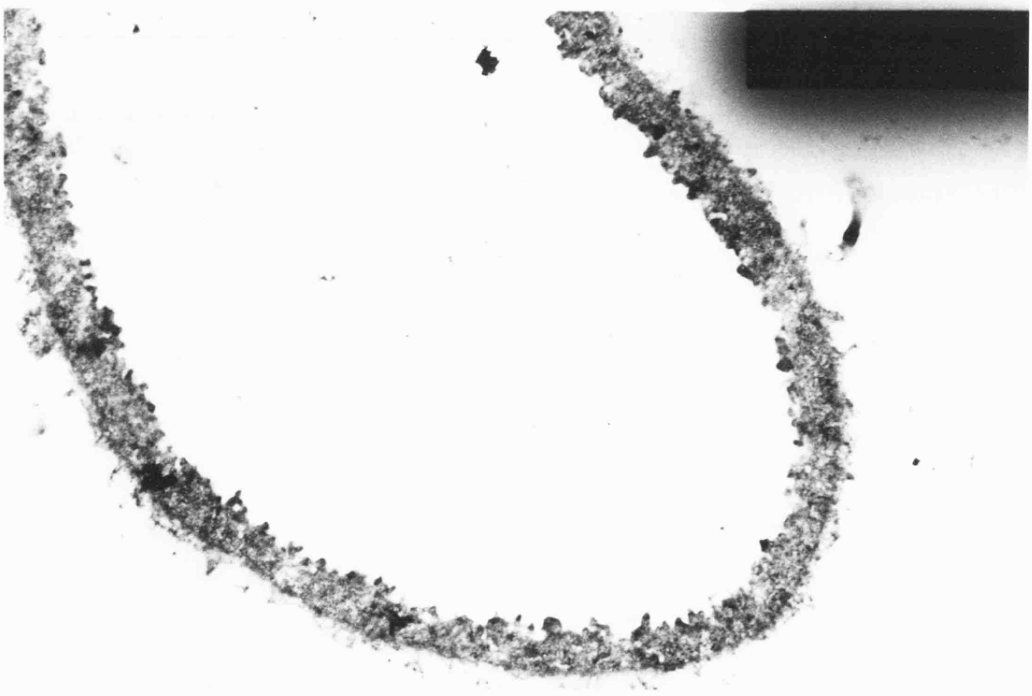


Scale: ⊢——⊣
1.0μm

FIGURE 3.33 SCANNING ELECTRON MICROGRAPHS OF THE SURFACE OF DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED BY a) METHOD A AND b) METHOD B.

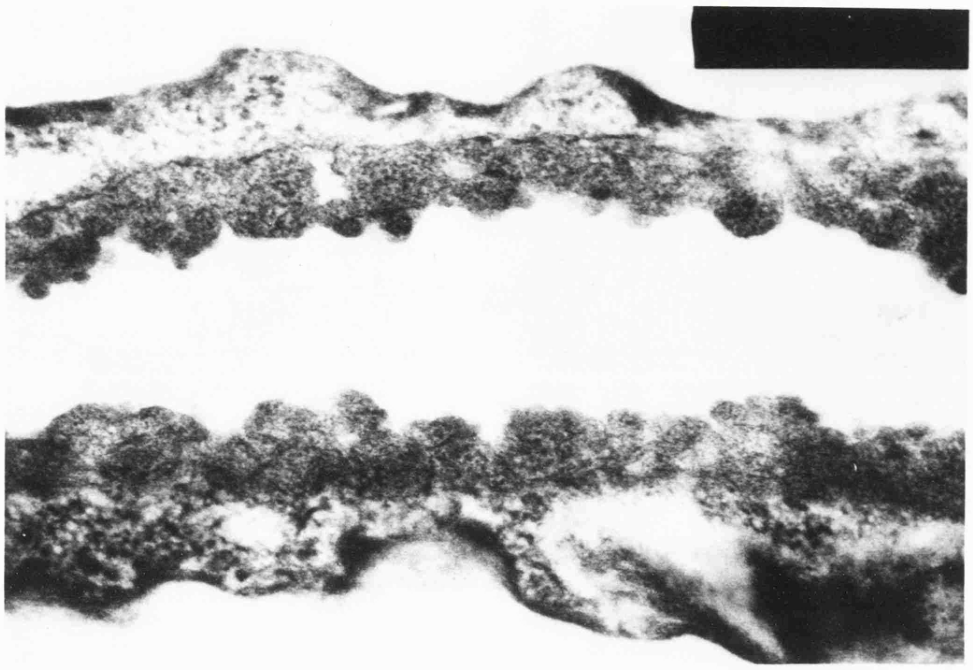


Magnification: x 100000 Scale: 0.1μm



Magnification: x 15000 Scale: 1.0μm

FIGURE 3.34 TRANSMISSION ELECTRON MICROGRAPHS OF A SECTION OF THE WALL OF DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED USING METHOD A.



Magnification: x 75000 Scale: 0.2μm

Magnification: x 15000 Scale: 1.0μm

FIGURE 3.35 TRANSMISSION ELECTRON MICROGRAPHS OF A SECTION OF THE WALL OF DOUBLE WALLED POLYPHTHALAMIDE MICROCAPSULES PREPARED USING METHOD B.

3.8 Permeability of Polyamide Films

The diffusion and permeability of solutes through a polymer may be determined from film permeability measurements and construction of a Barrer Plot (138), a method which has been used extensively elsewhere (68, 73). In order to assess the permeability characteristics of polyphthalamide membranes, the permeability of pilocarpine nitrate through films prepared by interfacial polymerisation was determined. The permeability of ethyl-4-aminobenzoate through such films was also investigated as an example of a solute whose interaction with polyamides is well characterised (67, 68, 73). For comparative purposes the permeability of both pilocarpine nitrate and ethyl-4-aminobenzoate through a commercially available non-oriented nylon 6 film was also determined.

3.8.1 Preparation of Polyphthalamide Films

Polyphthalamide films were prepared by an interfacial polycondensation technique. 60ml cyclohexane containing 0.01 moles phthaloyl chloride was placed in a 23cm diameter watch glass. 60ml of aqueous 0.4M piperazine containing 20ml 0.45M carbonate-bicarbonate buffer was introduced through a funnel under the cyclohexane layer. The film formed at the interface was of insufficient strength to support its own weight when removed from the reaction solutions. Consequently, after removing the polymer film formed initially a metal grid was placed in the aqueous solution and after approximately 30 seconds 'lifted' through the aqueous/organic interface, thus coating the grid with the freshly formed polymer.

3.8.2 Estimation of Polyphthalamide Film Thickness

Due to the fragility of the polyphthalamide films formed and their water swollen nature it was not possible to determine film thickness using conventional methods such as a micrometer. The approximate thickness of polyphthalamide films mounted on a metal grid was therefore determined using a vernier microscope. Five measurements were made on each of five samples which gave values ranging between 20μm and 140μm. The mean values for each sample were 75μm, 90μm, 50μm, 75μm and 80μm which gave an overall mean of approximately 75μm and a sample standard deviation of 40μm. The large variation in film thickness was attributed to difficulty in coating the grid with a uniform layer of polymer and also in focussing the vernier microscope on the edge of the film. The values of film thickness can therefore be considered to be only approximate.

3.8.3 Estimation of Non-Oriented Nylon 6 Film Thickness

The thickness of non-oriented nylon 6 film (Carron Grade 77c) was estimated using a micrometer. Five measurements were made on each of five samples of dry film and the mean thickness estimated to be 21.8μm (S.D. = 2.6μm). It has been shown previously that wet and dry film thicknesses are not significantly different for nylon 6 membranes (68).

3.8.4 Measurement of Permeability

General Method

Permeability cells of an all glass construction were used, which consisted of a donor and receptor compartment separated by a polyamide film as shown in Figure 3.36. Each compartment had a capacity of 50ml and the aperture diameter was 3.5cm. The two compartments were tightly clamped together and in the case of the polyphthalamide film, the film supported by the metal grid was clamped between the two compartments. The donor and receptor solutions and the assembled apparatus were equilibrated to 32^{o}C in a water bath. Immediately before the start of an experiment the permeability cell was quickly dismantled and a freshly prepared polyphthalamide film or a nylon 6 film clamped between the cells and reimmersed in the water bath. The solutions were added simultaneously into their respective compartments at the same rate to avoid undue distension of the membrane. This was achieved by adding the solutions via burettes positioned above the donor and receptor compartments. The solutions in each compartment were stirred by an all glass paddle stirrer at 200 rpm shaft speed. It was not possible to use a faster stirring speed due to the fragility of the membrane. The donor solution consisted of a solution of the appropriate solute in buffer, and the receptor solution was the buffer solution without solute. Samples were removed from the receptor solution at timed intervals for assay. The sample volume for the assay of pilocarpine nitrate by liquid scintillation counting was 1.5ml, and 1.0ml for the assay of ethyl-4-aminobenzoate. The mass of solute removed in each sample was corrected for when calculating the permeation data.

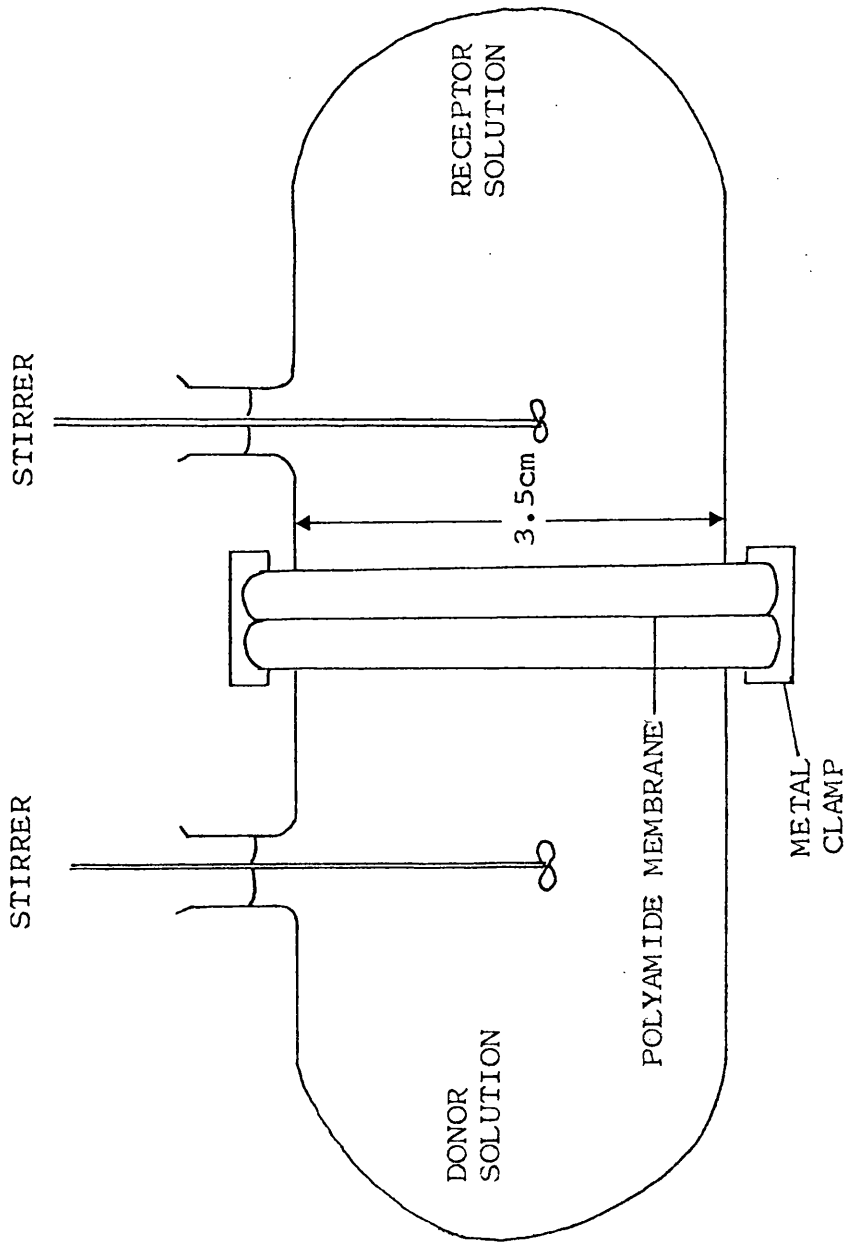


FIGURE 3.36 SCHEMATIC REPRESENTATION OF THE PERMEABILITY CELL.

Permeability of Polyphthalamide Film

The permeability of pilocarpine nitrate through polyphthalamide film is illustrated in Figure 3.37 and the data are given in Table 3.27. The donor solution used was 1.85×10^{-2}M pilocarpine nitrate in pH7.4 isotonic phosphate buffer also containing tritiated pilocarpine. It can be seen from Figure 3.37 that the permeation of the pilocarpine nitrate was rapid, the donor and receptor solutions reaching equilibrium in approximately 120 minutes. Comparison of the data for two separate determinations shows good reproducibility.

The permeation of ethyl-4-aminobenzoate through polyphthalamide film is represented in Figure 3.38. The two donor solutions investigated were (a) 3.02×10^{-3}M ethyl-4-aminobenzoate in pH 1.0 Clark Lub's buffer and (b) 3.01×10^{-3}M ethyl-4-aminobenzoate in pH 7.4 Sorensens phosphate buffer. Using a pKa value of 2.57 (67) the solute would be approximately 97% and 1.5×10^{-3}% in the ionised form at the two respective pHs. It has been reported that ethyl-4-aminobenzoate degrades rapidly at high temperatures and low pH (139). However, at the temperature and pH of this experiment the degradation of the solute over the time period studied was considered negligible. Examination of Figure 3.38 and Table 3.27 show that there is no apparent difference in the permeation of ionised and unionised ethyl-4-aminobenzoate through the polyphthalamide film. Again equilibrium was attained within 120 minutes.

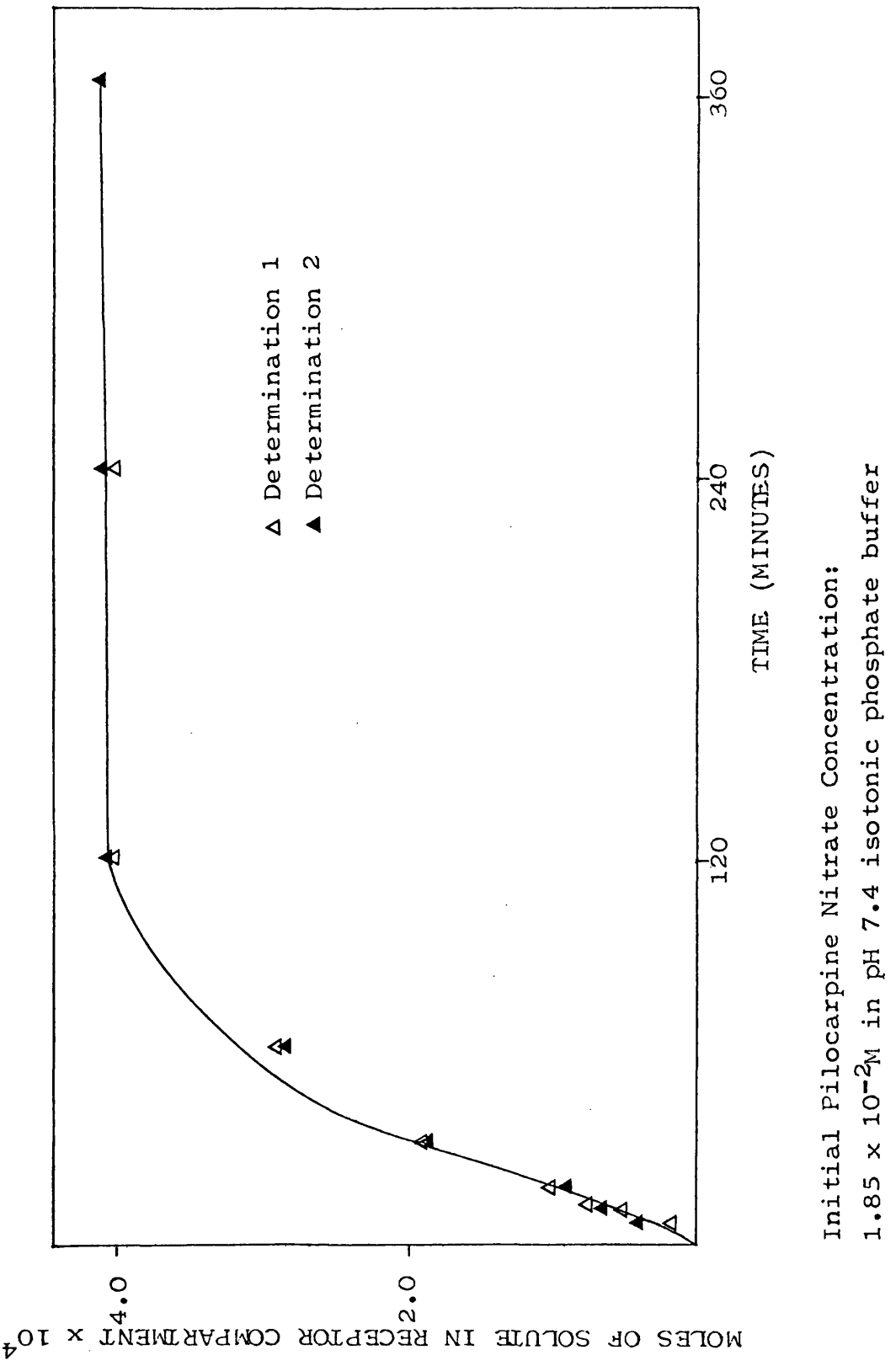
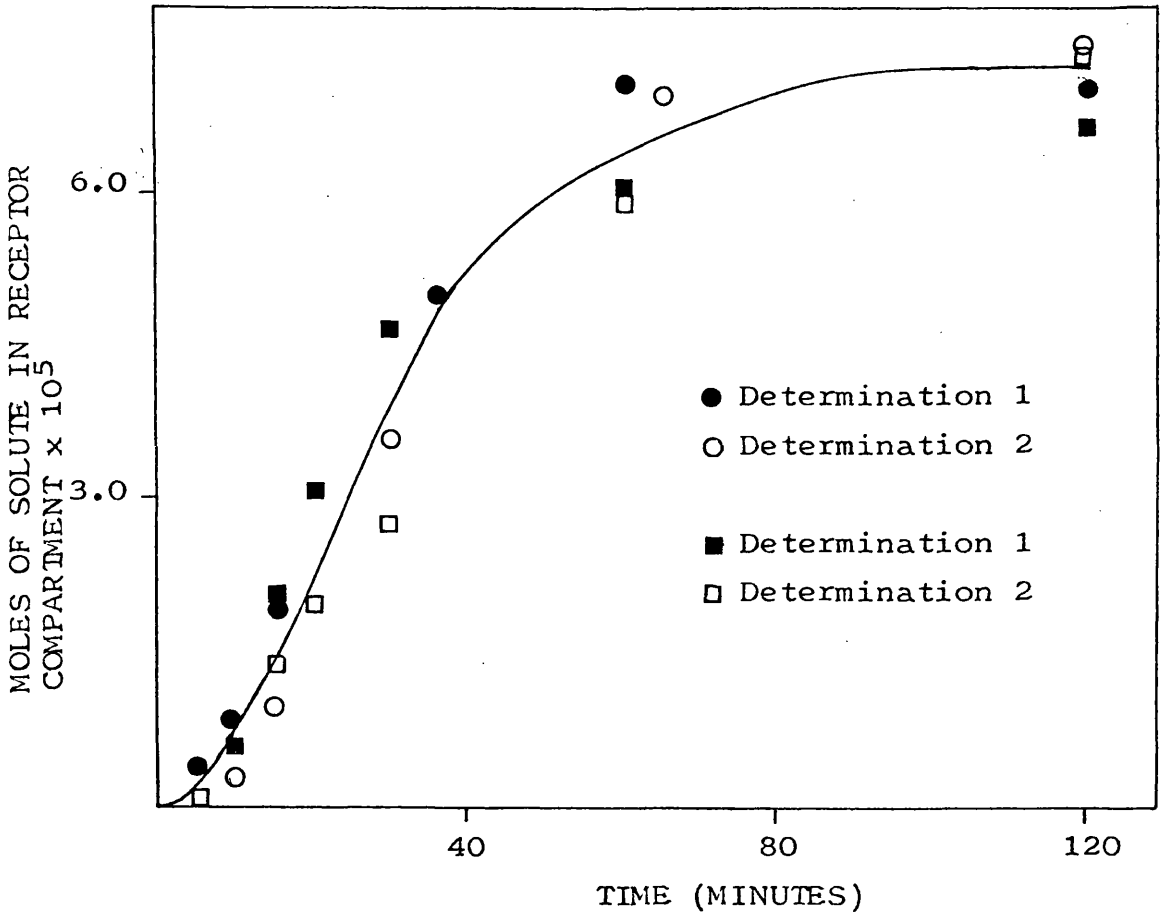


FIGURE 3.37 THE PERMEATION OF PILOCARPINE NITRATE THROUGH POLYPHTHALAMIDE FILM (approximate thickness 75μm) AT 32°C.



● ○ Ethyl-4-aminobenzoate, initial concentration 3.02×10^{-3}M in pH 7.4 isotonic phosphate buffer.

■ □ Ethyl-4-aminobenzoate, initial concentration 3.01×10^{-3}M in pH 1.0 buffer.

FIGURE 3.38 THE PERMEATION OF ETHYL-4-AMINOBENZOATE THROUGH POLYPHTHALAMIDE FILM (approximate thickness 75μm) AT 32°C.

Initial Donor Solution.	Moles of Solute Present in Receptor Compartment.					
	1.85×10^{-2}M Pilocarpine Nitrate in pH 7.4 Buffer.		3.02×10^{-3}M Ethyl-4-Amino-benzoate in pH 7.4 Buffer		3.01×10^{-3}M Ethyl-4-Amino-benzoate in pH 1.0 Buffer.	
	Determination 1	Determination 2	Determination 1	Determination 2	Determination 1	Determination 2
Time (minutes)						
5	1.61×10^{-5}	3.62×10^{-5}	4.04×10^{-6}	0.90×10^{-6}	0.75×10^{-6}	0.75×10^{-6}
8	4.77×10^{-5}					
10	7.39×10^{-5}	6.38×10^{-5}	7.93×10^{-6}	3.14×10^{-6}	9.63×10^{-6}	6.17×10^{-6}
15	11.13×10^{-5}	9.22×10^{-5}	20.36×10^{-6}	9.99×10^{-6}	21.07×10^{-6}	14.45×10^{-6}
20					31.45×10^{-6}	20.02×10^{-6}
30	19.64×10^{-5}	19.18×10^{-5}	50.30×10^{-6}	36.52×10^{-6}	47.71×10^{-6}	27.09×10^{-6}
60	29.83×10^{-5}	28.90×10^{-5}	72.01×10^{-6}	70.05×10^{-6}	62.01×10^{-6}	60.50×10^{-6}
120	43.20×10^{-5}	43.00×10^{-5}	70.66×10^{-6}	75.70×10^{-6}	67.42×10^{-6}	74.05×10^{-6}
240	43.10×10^{-5}	43.50×10^{-5}				
360	44.10×10^{-5}					

TABLE 3.27 THE PERMEABILITY OF POLYPHTHALAMIDE FILM (Approximate Thickness 75μm) TO PILOCARPINE NITRATE AND IONISED AND UNIONISED ETHYL-4-AMINOBENZOATE AT 32°C.

Permeability of Non-Oriented Nylon 6 Film

The data presented in Figure 3.39 illustrate the permeation of pilocarpine nitrate and ionised and unionised ethyl-4-aminobenzoate through non-oriented nylon 6 film. The donor and receptor solutions were as described for the permeability of polyphthalamide film. As can be seen from Figure 3.39 and Table 3.28 the pilocarpine nitrate and ionised ethyl-4-aminobenzoate did not permeate the nylon 6 film within 5 hours. The observed lag time for the permeation of unionised ethyl-4-aminobenzoate was approximately 40 minutes. The permeability coefficient of unionised ethyl-4-aminobenzoate through non-oriented nylon 6 film was calculated using Equation 3.4.

$$\frac{dC_R^s}{dt} = \frac{PAC_D^s}{lV_R} \qquad \text{(equation 3.4)}$$

where C_R^s is the receptor solution concentration

C_D^s is the donor solution concentration

V_R is the volume of the receptor compartment

A is the cross sectional surface area

l is the film thickness

P is the permeability coefficient

Using the slope of the Barrer plot $(\frac{dC^s_R}{dt})$ the value of the permeability coefficient was calculated to the 1.06×10^{-12} $M^2\ sec^{-1}$.

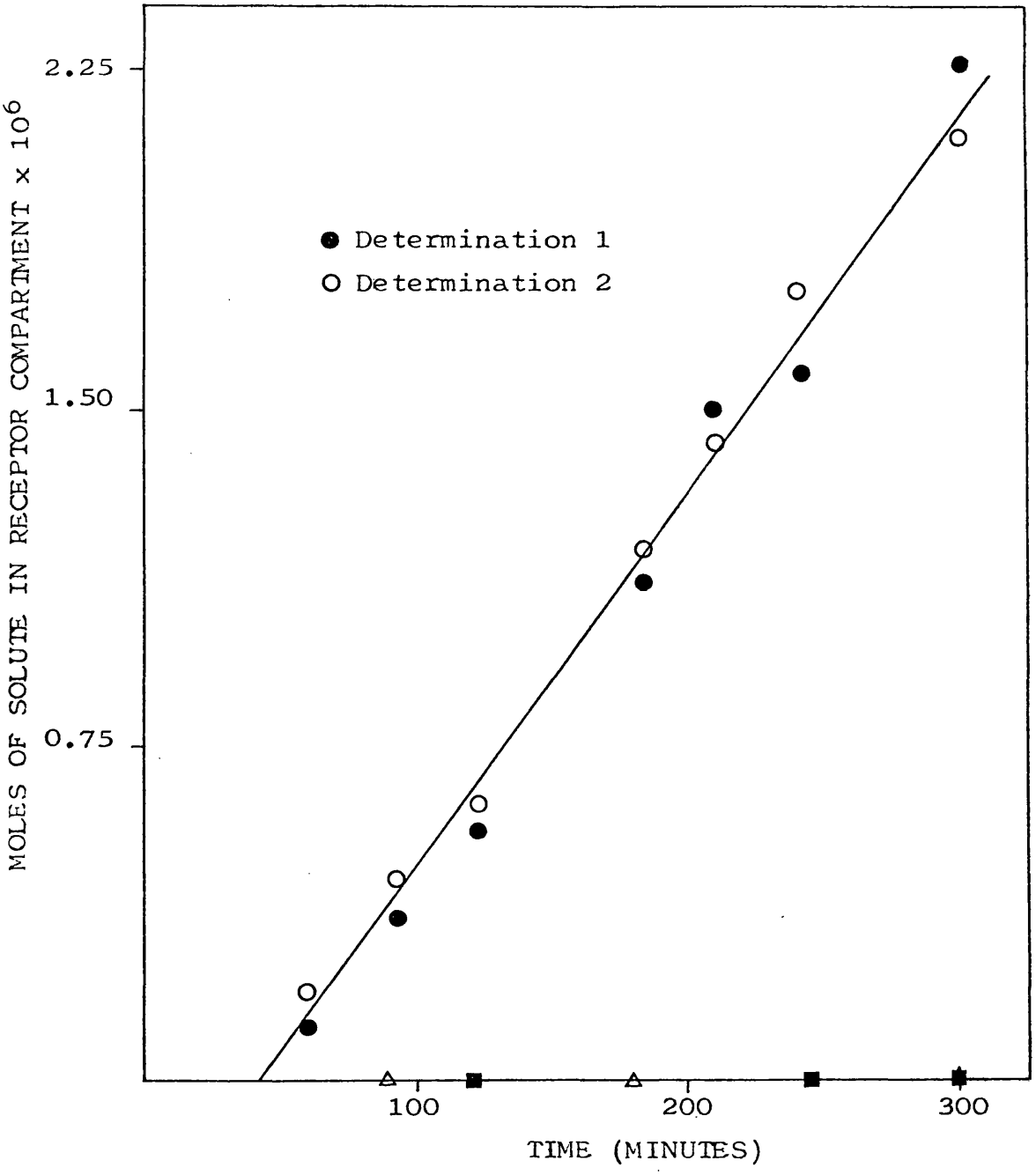


FIGURE 3.39 THE PERMEATION OF PILOCARPINE NITRATE IN pH 7.4 BUFFER (△), ETHYL-4-AMINOBENZOATE IN pH 7.4 BUFFER (●○) AND ETHYL-4-AMINOBENZOATE IN pH 1.0 BUFFER (■), THROUGH NON-ORIENTED NYLON 6 FILM OF THICKNESS 21.8μm, AT 32°C.

Initial Donor Solution.	Moles of Solute Present in Receptor Compartment.					
	1.85×10^{-2}M Pilocarpine Nitrate in pH 7.4 Buffer.		3.02×10^{-3}M Ethyl-4-Amino-benzoate in pH 7.4Buffer.		3.01×10^{-3}M Ethyl-4-Amino-benzoate in pH 1.0 Buffer.	
	Determination 1	Determination 2	Determination 1	Determination 2	Determination 1	Determination 2
Time (minutes)						
30	NO SOLUTE DETECTABLE	NO SOLUTE DETECTABLE	0.00	0.00	NO SOLUTE DETECTABLE	NO SOLUTE DETECTABLE
60			1.21×10^{-7}	2.01×10^{-7}		
90			3.59×10^{-7}	4.76×10^{-7}		
120			5.33×10^{-7}	6.07×10^{-7}		
180			11.05×10^{-7}	10.39×10^{-7}		
210			14.09×10^{-7}	13.69×10^{-7}		
240			15.60×10^{-7}	17.83×10^{-7}		
300			22.71×10^{-7}	21.16×10^{-7}		

TABLE 3.28 THE PERMEABILITY OF NON-ORIENTED NYLON 6 FILM (THICKNESS 21.8μm) TO PILOCARPINE NITRATE AND IONISED AND UNIONISED ETHYL-4-AMINOBENZOATE AT 32°C.

3.9 Polyphthalamide Microcapsules - In Vivo Studies

The effect of microencapsulated pilocarpine nitrate on the pupil diameter of the rabbit was investigated. A suspension of polyphthalamide microcapsules for instillation into the eye was prepared as follows. Polyphthalamide microcapsules were frozen in liquid nitrogen and freeze dried for a period of five hours. These were then immersed in an aqueous solution of 7.38×10^{-2}M pilocarpine nitrate to give a concentration of microcapsules of approximately 25% v/v. The suspension was allowed to equilibrate for 12 hours after which time 0.05ml of this suspension was placed in the conjunctival cul-de-sac of the left eye of each of two rabbits using a replicating pipette. Determination of the miosis induced by the pilocarpine nitrate was made by measuring the pupil diameter of the left eye using a plastic rule prior to and at timed intervals after dosing. The rabbits were maintained under constant lighting conditions.

After a rest period of one week this was repeated using a 7.38×10^{-2}M aqueous solution of pilocarpine nitrate. The left eye of the same rabbits was again used. Figure 3.40 shows the effect of both preparations on the pupil diameter of both rabbits. In the case of both the pilocarpine nitrate solution and the microcapsule suspension in rabbit A and rabbit B the diameter reached a minimum at approximately 30 minutes post instillation. The pupils returned to their original diameter within the accuracy of measurement after approximately 180 minutes.

The dwell time of polyphthalamide microcapsules in the left eye of each of four rabbits was determined using a gamma camera technique.

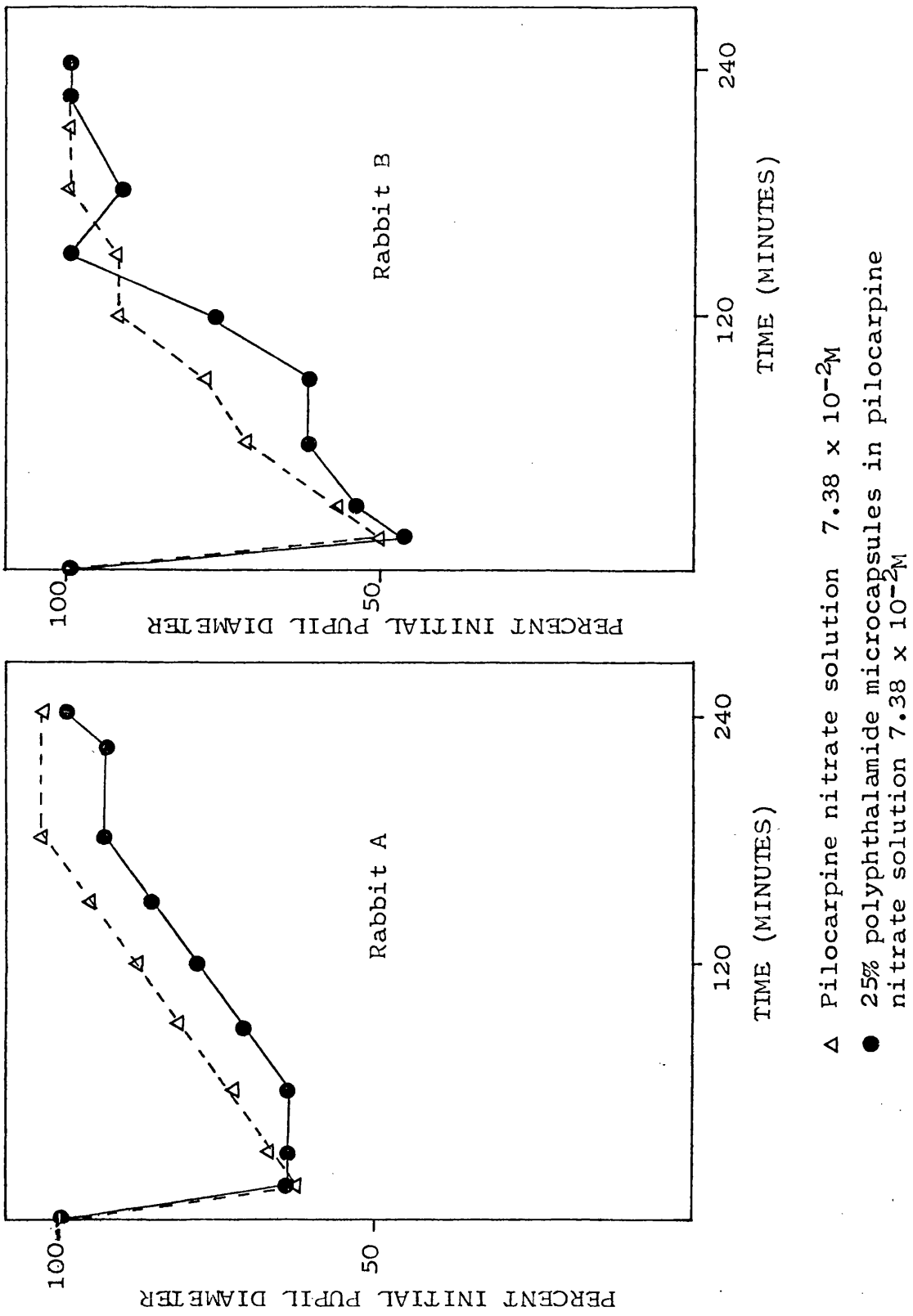


FIGURE 3.40 THE EFFECT OF POLYPHTHALAMIDE MICROCAPSULES ON THE MIOSIS INDUCED BY PILOCARPINE NITRATE IN THE RABBIT EYE.

Polyphthalamide microcapsules containing ^{99M}Tc labelled albumin, a gamma ray emitter, were prepared as in Section 3.2. The labelled albumin was dissolved in the aqueous phase prior to encapsulation to give a concentration of 2mg per ml and an activity of approximately 20 mCi per ml. After preparation the suspension was frozen in liquid nitrogen and freeze dried. Sufficient water was then added to the dried microcapsules to form a suspension of approximate concentration 5% v/w (determined by Coulter Counter). 0.05ml of the suspension was instilled into the left eye of the rabbit and the animal located such that the eye was positioned for observation by the Maxi Camera 11 which was fitted with a medium pin hole collimator and spacer. Images of total gamma emission over 15 second periods were recorded continuously for 15 minutes and discontinuously for up to approximately 90 minutes. Experiments with fluoresceinated albumin showed that the albumin remains inside the microcapsules (see Section 4.3).

As a comparison an estimation of the rate of clearance of labelled albumin in aqueous solutions was also made. 0.05ml of aqueous ^{99M}Tc labelled albumin solution of concentration 10mg per ml and an activity of 1mCi per ml was instilled into the left eye of the rabbits used above 24 hours prior to dosing with the microcapsules. The rabbit was located such that the left eye was positioned for observation by the Maxi Camera 11 and images of gamma emission over a 15 second period were recorded continuously for 15 minutes.

From the images obtained regions of interest were drawn around the orbit of the eye and the tear duct. The activity remaining in these areas at given times was calculated. Corrections were made

for background count and for technetium decay using Equation 3.5.

$$N_o = N_e^{\lambda t} \qquad \text{(equation 3.5)}$$

where $\lambda = \frac{0.693}{6.0}$

t = time in hours

6.0 is the half life of technetium in hours.

Figure 3.41 represents the difference in dwell time in the rabbit eye between polyphthalamide microcapsules containing ^{99M}Tc labelled albumin and an aqueous solution of ^{99M}Tc labelled albumin. The aqueous albumin solution was cleared rapidly from the eye, reaching approximately 10% of the instilled mass within 15 to 20 minutes. Approximately 60% of the instilled mass of microcapsules however was still present in the eye after 90 minutes. Photographs of selected gamma camera images are shown in Figures 3.42 to 3.44. Figure 3.42 shows the distribution of labelled albumin solution in the eye of rabbit A as a function of time whereas Figure 3.43 shows the distribution of polyphthalamide microcapsules containing labelled albumin also in the eye of rabbit A as a function of time. In both Figures, Plates 1, 2 and 3 are the distribution at times 0,15 seconds and 45 seconds respectively. In Figure 3.42 Plate 4 gives the distribution at 7 minutes 45 seconds and in Figure 3.43 Plate 4 gives the distribution at 6 minutes 45 seconds. The shade of colour is a function of the intensity of activity, white being the greatest concentration of activity and blue the least. It may be seen from Figure 3.42 that after 15 seconds the albumin solution is beginning to drain through the lachrymal duct and that by 7 minutes 45 seconds drainage is

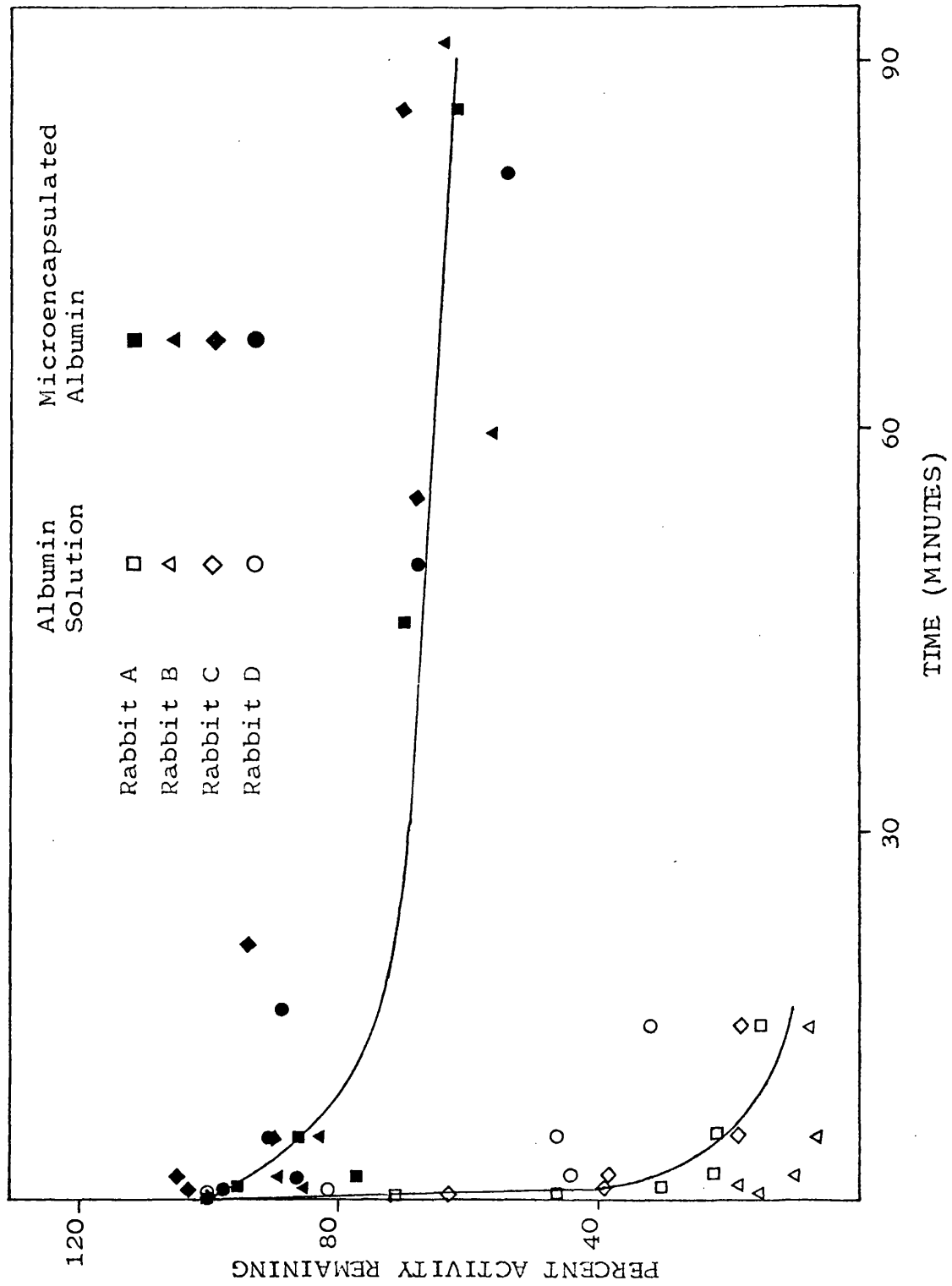


FIGURE 3.41 THE DWELL TIME OF ALBUMIN AND POLYPHTHALAMIDE MICROENCAPSULATED ALBUMIN IN THE RABBIT EYE.

almost complete. In contrast the series of images in Figure 3.43 showing the distribution of polyphthalamide microcapsules containing albumin indicate that the microcapsules collect over the inner portion of the eye and form a 'horse-shoe'. They are not evenly dispersed over the surface and there are no signs of drainage occurring. Figure 3.44 shows the distribution of polyphthalamide microcapsules containing labelled albumin at longer time periods. Plates 1, 2 and 3 are the left eye of rabbits A, B and C at 45 minutes, 30 minutes and 20 minutes respectively. In all cases drainage into the lachrymal duct has started to occur although a large proportion of microcapsules are still present on the surface of the eye.

FIGURE 3.42 THE CLEARANCE OF ^{99M}Tc LABELLED ALBUMIN SOLUTION FROM THE RABBIT EYE.

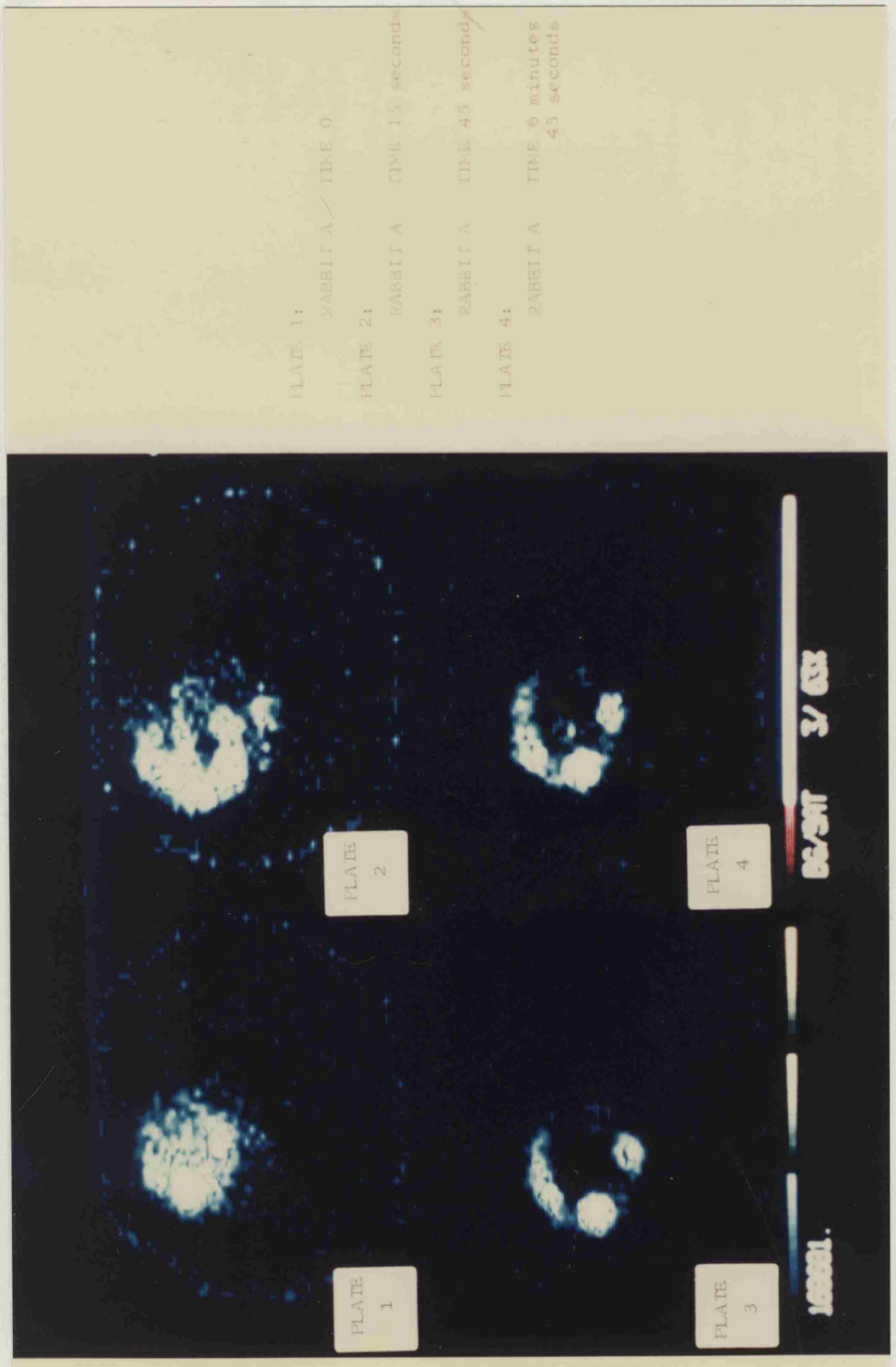


FIGURE 3.43 THE CLEARANCE OF POLYPHTHALAMIDE MICROCAPSULES CONTAINING ^{99M}Tc LABELLED ALBUMIN SOLUTION FROM THE RABBIT EYE.

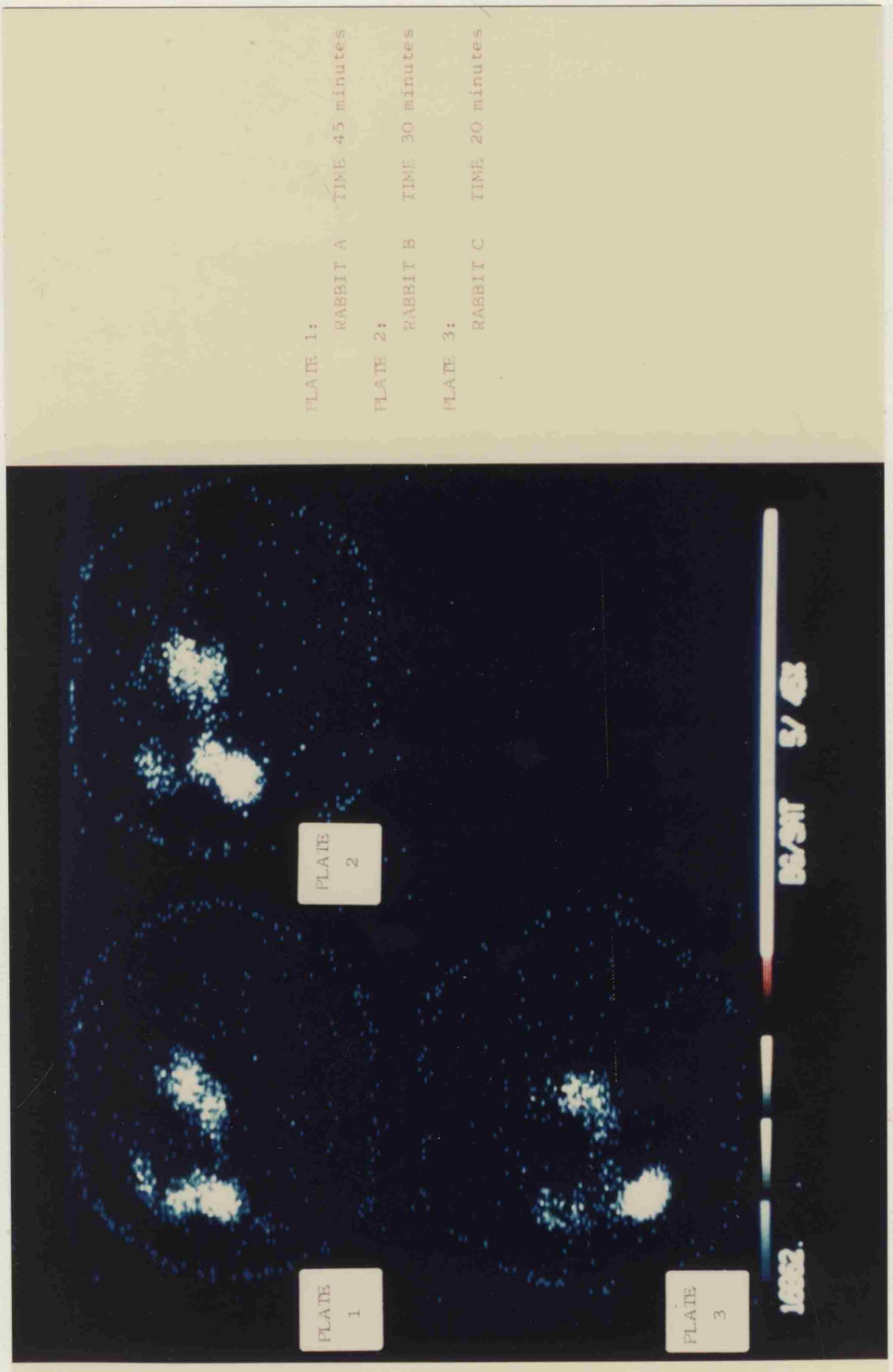


FIGURE 3.44 THE CLEARANCE OF POLYPHTHALAMIDE MICROCAPSULES CONTAINING ^{99M}Tc LABELLED ALBUMIN SOLUTION FROM THE RABBIT EYE AT LONG TIME PERIODS.

DISCUSSION

4.1 Preparation and Properties of Nylon 6.10 and Polyphthalamide Microcapsules

Previous attempts to prolong the actions of drugs in the treatment of glaucoma have involved the use of viscolysers or large reservoir devices such as the Ocusert (see Section 1.1.3.4). An alternative approach to controlling the release of drugs to the eye is to prepare a suspension of drug containing microcapsules which, on ophthalmic administration, will slowly release their contents. This concept is based on literature reports concerning the preparation and properties of microcapsules which show that:

1) Microcapsules containing a variety of drug substances can be readily prepared using a number of techniques (46-50, 90, 99, 106, 107, 140).
2) The release of drugs from microcapsules can be controlled (99, 122, 141, 142).
3) Microcapsules can be prepared having a mean diameter of less than 20μm, a size which is considered suitable for the administration of particles to the eye (29, 87, 143, 144, 145).
4) Microcapsules prepared by some techniques are flexible (29), a property which may reduce irritations caused by particles when administered to the eye.

Of the many methods available for the preparation of microcapsules (Section 1.2.2) few are designed to encapsulate drugs dissolved or dispersed in an aqueous core. Coacervation for example is used solely for the encapsulation of lipophilic molecules whereas physical methods of microencapsulation such as the Wurster Process generally result in the encapsulation of solid particles. Of the methods used for preparing microcapsules containing an aqueous core, there have been several reports concerning the interfacial polymerisation

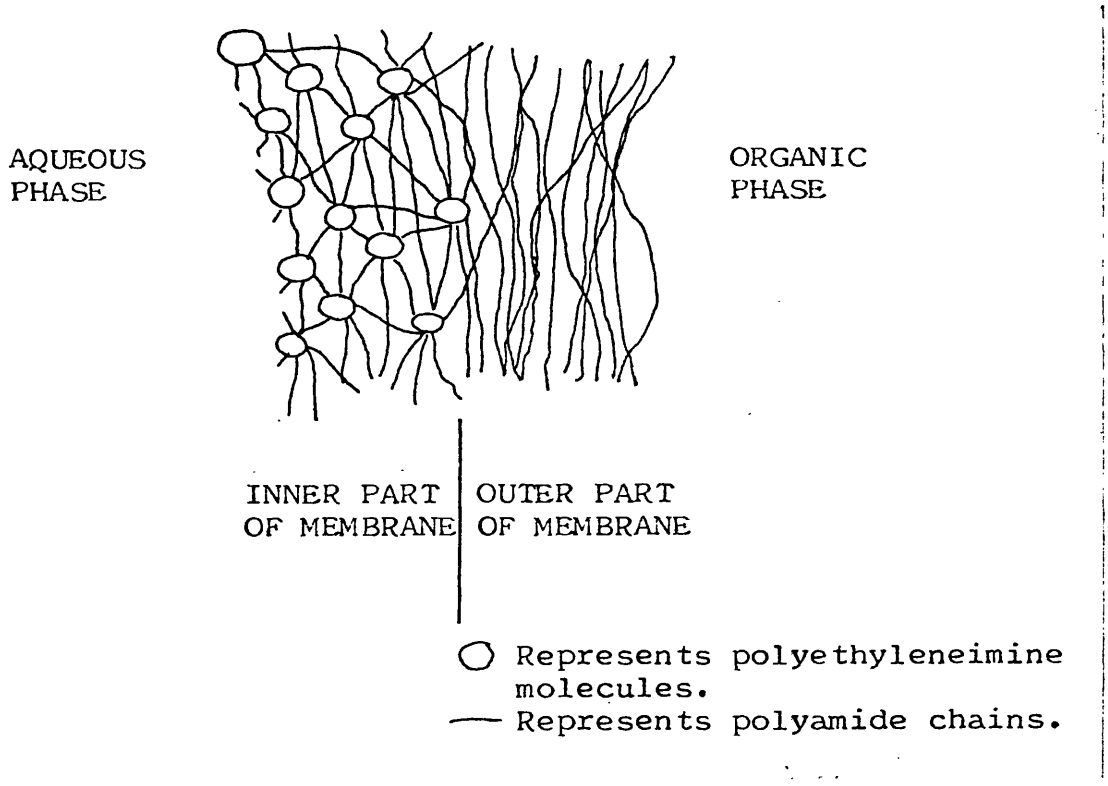
technique. Using this technique, some of the more frequently reported polymers utilised as wall materials are the polyamides (87-90, 92-112, 118-120, 126-129). Other examples include polyurethane and polyphenolester (146, 147). From the literature it appears that polyamide microcapsules can be readily prepared and therefore this type of microcapsule was selected for study in this investigation. The majority of reports concerning the preparation of this type of microcapsule are based on the original method described by Chang and others (29) for the preparation of nylon 6.10 microcapsules. An outline of this method is given in Section 1.5.2.1. Attempts to prepare microcapsules in this study using this method were however unsuccessful and resulted only in the formation of what appeared to be small particles of polymer and emulsion droplets. In the successful preparation of polyamide microcapsules, other workers have previously modified the technique of Chang and others. Such modifications include the microcapsule harvesting technique, the method of emulsion formation, the composition of the reactant solutions and the reaction conditions (87, 89, 92, 93, 95, 98, 99, 100, 110). These are described fully in Section 1.5.2. Attempts to modify the initial method of preparation based on these reports were unsuccessful in this investigation in producing microcapsules.

It was noted that in Chang's original method of preparing nylon 6.10 microcapsules the aim was to encapsulate haemoglobin (29). Haemoglobin contains amino groups which during polymerisation react with the linear polymer chains thereby crosslinking the polymer and becoming incorporated into the microcapsule membrane (94). This may result in the formation of a more stable membrane giving rise to the successful formation of microcapsules. Other reports have indicated that proteins such

as albumin are also incorporated into the microcapsule walls by reaction of their amino groups with the acid chloride molecules in the polymer chains (120). It was suggested by Aisina and others (94) that polyethyleneimine could similarly be used to crosslink the linear chains of polymer forming a network as shown in Figure 4.1. It is expected that the microcapsule membranes with such a crosslinked

Figure 4.1

Schematic Representation of the Structure of Microcapsule Membranes prepared containing Polyethyleneimine



structure will be stronger than those prepared in the absence of crosslinking agents and therefore the inclusion of crosslinking agents such as haemoglobin or polyethyleneimine will result in an increased microcapsule yield. In this study the incorporation of 10% w/v polyethyleneimine in the aqueous phase used in the hitherto unsuccessful methods of microencapsulation, resulted in the successful formation of nylon 6.10 microcapsules (Section 3.11).

This contrasts to the work of Koishi and others (87) and Shigeri and others (89) who successfully prepared microcapsules using an interfacial polymerisation technique in the absence of any crosslinking agents. The methods used however resulted in the preparation of relatively small microcapsules of approximately 2μm diameter, and no indication of the yield obtained was given.

In order to instill solid particles in the eye without irritation it has been suggested that their diameter should be less than 20μm (135). Conventionally microcapsules are considered to be of a size ranging from a few micrometers in diameter to a few millimeters (31) although the diameters of those prepared by interfacial polymerisation techniques are usually in the range 2.0μm (89) to 80μm (29). The microcapsules initially prepared in this study had diameters of approximately 200μm. There are a number of parameters which are known to influence the size of microcapsules prepared by interfacial polymerisation. These are primarily factors which affect emulsion droplet size and emulsion stability. With regard to emulsion droplet size, it is expected that an increase in the total shear in an emulsion system brought about by an increase in stirring speed, a change in the shape of container or a change in the type of stirrer, will result in a decrease in the size of the emulsion droplets formed (148). In a microencapsulation system, this will in turn result in a reduction in microcapsule size. For example, Koishi and others (87) reported that in the preparation of polyphthalamide microcapsules, increasing the stirring speed from 237 rpm to 1,100 rpm reduced the microcapsule size from 8.55μm to 2.45μm. Chang and others (29) also reported that for an increase in stirring speed from '1' to '5' there was a reduction in microcapsule

size from 80μm to 30μm. A similar effect was found in this investigation in which the diameters of nylon 6.10 microcapsules decreased from approximately 200μm to 20μm with a corresponding increase in stirring speed from approximately 500 rpm to 2,000 rpm (Section 3.1.1.2). There have been no reports in the literature concerning the effect of container shape on microcapsule size. However, several authors have reported the effect of mechanism of emulsion formation. Chang and others (29) for example found that the minimum diameter of microcapsules which could be prepared using a magnetic stirrer was approximately 20μm, whereas smaller microcapsules of about 2μm diameter could be prepared using an homogeniser. Of the methods of emulsification investigated in this study, only an overhead stirrer motor connected to a paddle stirrer was found to produce satisfactory microcapsules. This conflicts with the use of a magnetic stirrer and homogeniser in the successful preparation of microcapsules reported by Chang and others (29) and may arise due to differences in stirring speed. The magnetic stirrer used in this investigation may have been of insufficient speed to form a stable emulsion, or alternatively any microcapsules formed may have been too large to maintain their integrity. Conversely the emulsions formed by the ultrasonic probe and homogeniser may have been of too small a droplet size for microcapsule formation or perhaps the extreme shear in the system caused the disintegration of already formed microcapsules.

The effect of surfactant concentration on microcapsule size has been shown by Koishi and co-workers (87) in the preparation of polyphthalamide microcapsules. It was reported that with an increase in surfactant (Span 85) concentration from 5 to 15% v/v,

the mean volume diameter of the microcapsules formed decreased from 3.34μm to 3.16μm. Similarly, in the preparation of nylon 6.10 microcapsules Chang and others (29) found that on increasing the Span 85 concentration from 1 to 5% v/v the mean microcapsule diameter decreased from approximately 80μm to 40μm. Above 5% v/v however increasing the surfactant concentration had no appreciable effect on microcapsule size. In this study, no effect of surfactant concentration on microcapsule size was observed. However, during the initial studies, observations of microcapsule size were made using an eye piece micrometer with which only gross changes in size could be detected. Also the effect of surfactant concentration may not have been observed since a limited range of concentrations were investigated. Similarly, no effect of temperature on microcapsule size was observed although it has been reported previously that an increase in temperature results in a reduction in microcapsule size (89).

Development of the preparation of nylon 6.10 microcapsules containing polyethyleneimine based on the factors affecting microcapsule size discussed above resulted in the successful preparation of nylon 6.10 microcapsules of approximately 20μm diameter. In agreement with the work of Chang and others (29) who concluded that it was not possible to prepare microcapsules with diameters below 20μm using a magnetic stirrer, attempts to prepare microcapsules below 20μm using the overhead stirrer in this investigation were unsuccessful.

From the various techniques published in the literature concerning the preparation and recovery of polyamide microcapsules prepared by

the interfacial polymerisation method, it is apparent that each technique has yielded microcapsules with differing properties. It is suggested therefore that properties such as yield, size, size distribution and appearance are characteristic only for a given microencapsulation system and will vary widely between experimental workers. In order to study the properties of microcapsules prepared by interfacial polymerisation, Chang and others (29) reported a method by which the formed microcapsules were transferred from the organic reaction mixture to an aqueous phase. This involved quenching polymerisation by pouring the reaction mixture into the mixed solvent system which was followed by centrifugation, washing in cyclohexane and transference to an aqueous Tween 20 solution prior to dispersion in saline. Other workers have used similar methods involving quenching in the appropriate solvent system followed by washing with various concentrations and volumes of Tween 20 solution and resuspending in sodium chloride (92), phosphate buffer solution (103), Tween 20 solution (87) and Polysorbate 20 solution (100). Tween 20 and Polysorbate 20 are believed to aid dispersion and prevent the microcapsules aggregating. Alternatively Mori and colleagues (110) collected microcapsules either by filtration or centrifugation followed by washing in ethanol prior to washing with water, whereas Luzzi and others (99) recovered their microcapsules either by a spray drying process or by flash evaporation. The reasons for the use of these different methods were not however given. In the preparation of nylon 6.10 microcapsules in this study it was found that quenching in cyclohexane in place of the mixed solvent system aided centrifugation due to the increased density difference between the microcapsules and the suspending medium. Furthermore, washing

in acetone prior to the aqueous Tween 20 solution used by Chang and others (29) removed the bulk of the hydrophobic cyclohexane from the surface of the microcapsules and therefore aided dispersion in the aqueous medium. Recovery of the microcapsules from the aqueous Tween 20 solution by centrifugation as described by Chang and others however, was variable and full recovery was not achieved. This is thought to be due to the viscosity of the Tween 20 solution, its similarity in density to that of the microcapsules and the presence of trace amounts of cyclohexane. Separation from the medium by filtration, as suggested by Mori and others (110) was similarly unsuccessful. Although filtration through sintered glass and membrane filters removed some excess liquid from the microcapsule suspensions, it was observed that the filters readily blocked and the remaining microcapsule slurry contained a large proportion of extracapsular water (approximately 70-80%v/v as determined by Coulter Counter). This high volume of extracapsular water is thought to arise due to blockage of the filter by the microcapsules or to the formation of a packed bed of microcapsules on the surface of the filter which prevents the passage of liquid. Similar handling difficulties were encountered in an attempt to prepare a column of microcapsules through which buffer solution could be passed in order to simulate the release of drug in the eye. Another possible explanation for the presence of the high water content following filtration is obtained from a consideration of the packing of spheres and the volume of voids between them. For example, the porosity of orthorhombic packed monodisperse spheres has been calculated to be 40% (149) where porosity is defined as the ratio of the open space volume to the combined open space and solid material volumes, expressed as a

percentage. For cubic packed spheres this value was reported to be 47%. Consequently, if during filtration, the microcapsules in the bulk of the suspension remained as spheres, it is unlikely that all the extracapsular water would be removed.

Rotary evaporation, drying over phosphorous pentoxide and evaporation to the atmosphere were similarly unsuccessful in removing the extracapsular water without destroying the integrity of the microcapsules. Based on the method described by Mori and others (110) however, it was considered that washing the microcapsules in acetone, a solvent miscible with both cyclohexane and water, might yield a product suitable for direct dispersion into water. Microcapsules recovered by this method were readily redispersed in water to yield unflocculated suspensions. Concentration of these aqueous suspensions could be achieved either by rotary evaporation or by freeze drying. Freeze drying and rotary evaporation were also carried out on the acetone and cyclohexane washed microcapsules following centrifugation. The latter technique was used in the measurement of release from polyphthalamide microcapsules and is discussed in Section 4.2.2. In order to maintain the integrity of their nylon 6.10 microcapsules on storage Chang and co-workers (29) dispersed the microcapsules in isotonic saline. Shiba and others (92) similarly used 0.9% sodium chloride solution, whereas Grunwald and Chang (103) reported 0.1M phosphate buffer as a suitable suspending solution. In this investigation however, water was found to be a suitable medium in which to maintain the microcapsules. No osmosis such as that described by Chang and others (29) was observed to occur on dispersion of the microcapsules in hypertonic and hypotonic solutions. This difference is possibly due to the absence of a large

percentage of high molecular weight material (such as haemoglobin), from the core of the microcapsules prepared in this work. Polyamide microcapsule walls are thought to be impermeable to high molecular weight solutes but permeable to those of low molecular weight (119). Microcapsules containing a large proportion of haemoglobin might therefore be expected to exhibit similar osmotic properties to those found in cells with a semi-permeable membrane such as red blood cells. The absence of high molecular weight solutes or a considerable reduction in their concentration in the microcapsules could therefore account for the lack of osmotic effect observed.

One property of microcapsule suspensions which has been studied by a number of workers is their size distribution. Various techniques have been used to assess this property for example, Takenaka and others (143) used a particle size analyser to determine the size of gelatin acacia microcapsules. The most frequently reported technique however, involves linear measurement of the diameter of microscope images of aqueous microcapsule suspensions. This technique was used by Nixon and Hassan (150) for determining the size of gelatin acacia microcapsules containing thiabendazole and by Takenaka and others (151) for determining the size of enteric coated microcapsules prepared by spray drying. This technique was also used by Shigeri and others (89), Jenkins and Florence (106) and by Koishi and colleagues (87) for determining the size distributions of nylon 6.10 and polyphthalamide microcapsules prepared by the interfacial polymerisation technique. An alternative method for determining the size and size distribution of microcapsules is the Coulter Counter technique. This method was previously reported by Luzzi and

co-workers (99) for sizing polyamide microcapsules and Morris and Warburton (53) for determining the size and size distribution of three ply walled water/oil/water microcapsules. In this investigation the Coulter Counter gave reproducible results for the determination of nylon 6.10 and polyphthalamide microcapsule diameters as shown in Table 3.14. Values of the median volume diameter of four batches of polyphthalamide microcapsules calculated using this method were 27.0, 26.0, 23.0 and 28.0μm (Table 3.6). Results obtained were confirmed by microscope observations using the Fleming Particle Size Analyser (Figures 3.5 and 3.6).

The factors which have been shown to affect the size distribution of microcapsules prepared by the interfacial polymerisation technique are the same as those affecting microcapsule size discussed above. The size distribution of the microcapsules will therefore be dependent upon emulsification and microencapsulation conditions and would be expected to vary considerably between authors. Koishi and others (87) reported their polyamide microcapsules to have diameters ranging from less than 1 to approximately 8μm, whereas the spray dried nylon 6.10 microcapsules prepared by Luzzi and colleagues had a size distribution of approximately 6 to 30μm. Using the Coulter Counter technique the median volume diameter of both the nylon 6.10 and polyphthalamide microcapsules formed in this study ranged between approximtely 5μm and 60μm. Within this range however the values were not distributed normally. This is similar to size distributions for 3 ply walled and gelatin coacervate microcapsules determined by other workers (144, 150). Chang and others (29) reported that the nylon 6.10 microcapsules prepared using an homogeniser in their investigation did not exhibit a normal

size distribution. The distribution obtained showed a skew towards the lower capsule sizes. In contrast, Takenaka and others (143) and Luzzi and others (99) reported that the size distributions of their microcapsules did obey the log normal distribution law. In the latter example however, the lower 30% of the population was not included in the analysis. The size distributions of the polyamide microcapsules prepared in this study were again found to be skewed toward the lower capsule sizes. This is thought to have arisen as a result of rupture of the larger microcapsules during preparation and recovery or possibly as a result of instability or incomplete encapsulation of the larger emulsion droplets.

It has been reported by Koishi and others (89) that microcapsule size is dependent upon the type of polymer formed at the interface and the concentration of the monomers used to form the polymer. That is, it is thought to be a function of the strength of the microcapsule membranes. For example, it was reported that the mean diameter of microcapsules prepared from 1,6 hexamethylenediamine and sebacoyl chloride was 5.90μm whereas the diameters of those prepared from piperazine and phthaloyl chloride under the same polymerisation conditions was 3.37μm. This is consistent with the findings in this study which showed that altering the nature and concentration of the monomers used in the interfacial polyermisation affected the microcapsule size. Polyphthalamide microcapsules prepared using 0.044M phthaloyl chloride and 0.4M piperazine were found to have a median volume diameter of 26.0μm, in comparison with 16.0μm for nylon 6.10 microcapsules prepared using 0.015M sebacoyl chloride and 0.4M 1,6 hexamethylenediamine.

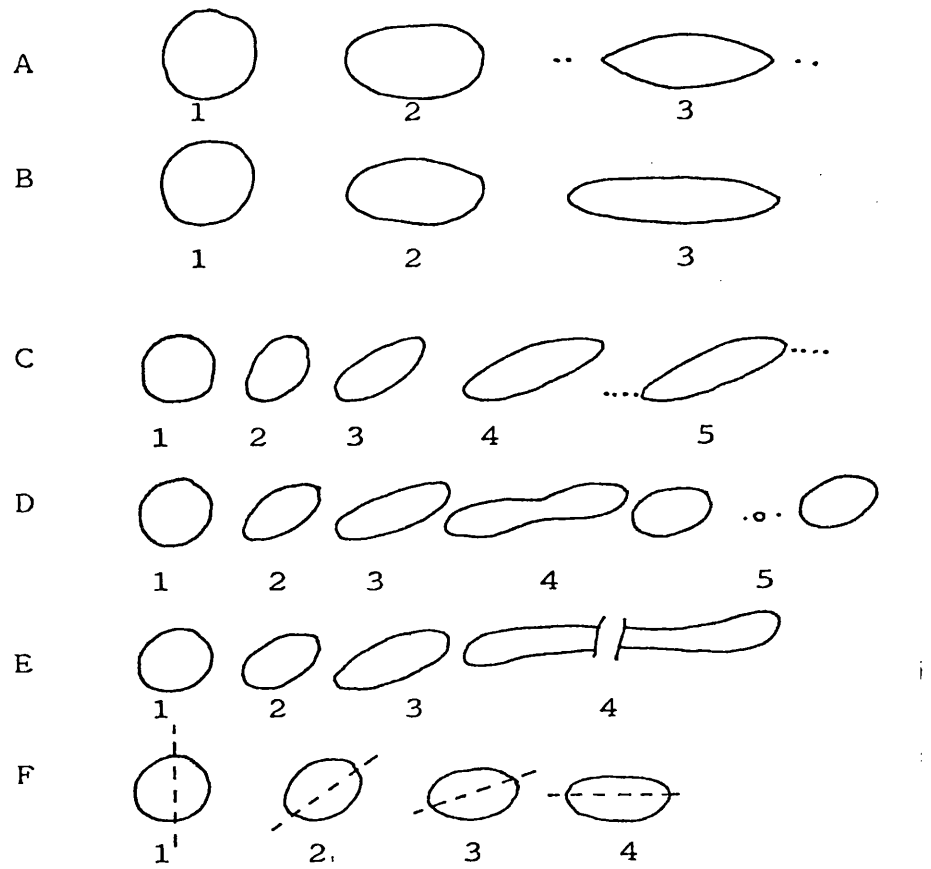
The method of determination of size distribution used also enabled estimation of the yield of microcapsules obtained. At present there is a paucity of information in the literature regarding the yield of microcapsules prepared by interfacial polymerisation and other techniques. In the preparation of nylon 6.10 and polyphthalamide microcapsules studied in this investigation it is expected that if all the aqueous phase is encapsulated, allowing for the relatively thin microcapsule walls, the yield of microcapsules should be approximately 7.5ml. The data in Table 3.8 (Section 3.3.3) shows that the estimated capsular volume for two batches of nylon 6.10 microcapsules following rotary evaporation was 0.23ml and 0.34ml. This is considerably lower than would be expected and may be due to incomplete encapsulation and/or rupture of the walls caused by centrifugation. Polyphthalamide microcapsules are believed to have stronger walls than nylon 6.10 (92) which may make them more resistant to centrifugation. However, batches of polyphthalamide microcapsules prepared using 0.015M phthaloyl chloride were found to have yields of only 0.53ml and 0.32ml, very little greater than that for nylon 6.10 microcapsules. In an attempt to increase microcapsule yield, the concentration of diacid chloride present was increased 3-fold. This resulted in a yield of approximately 5ml (Table 3.8). For four batches the yields ranged from 3.57ml to 5.85ml. This increase in yield may be due to an increase in the mass of polymer formed enabling more complete encapsulation to be achieved. A similar effect however, was not observed in the nylon 6.10 system when the concentration of sebacoyl chloride was increased 3-fold. This resulted only in the formation of what appeared to be an amorphous mass of polymer extending from the microcapsule surface into the organic phase. This is thought to be due to excess polymerisation in the bulk of the organic phase. The disparity

between the two systems is believed to arise as a result of differences in the partition coefficients of the two diamines for the organic phase. 1,6 hexamethylenediamine is reported to have a greater affinity for the organic phase than has piperazine (87). Alternatively, the disparity may be due to differences in the optimal polymerisation conditions for the two combinations of monomers. (see Section 1.5.1.2).

Differences between the nylon 6.10 and polyphthalamide microcapsule systems are also seen in the appearance of the microcapsules (Figures 3.1-3.4). The appearance of polyamide microcapsules prepared by interfacial polymerisation techniques has been the subject of several reports in the literature (29, 87, 92, 90). It has been reported that, in general, such microcapsules are mainly spherical with clear interiors. Exceptions to this are the spray dried polyamide microcapsules prepared by Luzzi and others (99) and the microcapsule suspensions prepared by Chang and others (29) using an homogeniser, which were found to contain a significant number of irregularly shaped capsules. Photographs and photomicrographs of the nylon 6.10 microcapsules prepared in this investigation (Figures 3.1, 3.2) are very similar to those shown by other workers (29, 90, 92, 87). The microcapsules formed were again found to be mainly spherical with clear interiors. In contrast, however, the polyphthalamide microcapsules were more irregular in shape although the interiors were clear (Figures 3.3, 3.4). Observations of the shapes obtained from the deformation of emulsion droplets in plane hyperbolic and shear flow have been reported by Rumscheidt and Mason (115) and are illustrated in Figure 4.2.

Figure 4.2

The Deformation and Burst of Emulsion Droplets in Plane Hyperbolic (A&B) and Shear Flow (C,D,E&F) (115)



These shapes are typical of those found in the polyphthalamide microcapsule suspensions which are therefore thought to arise as a result of coalescence or deformation of emulsion droplets as polymerisation occurs. Similarly shaped microcapsules may also be formed in the nylon 6.10 system. Due to the weaker membrane of the nylon 6.10 capsules however (92) these may subsequently rupture to form new spherical emulsion droplets. In the presence of continuing polymerisation these may then form new spherical microcapsules. In the absence of polymerisation however, the emuls ion droplets will remain as unencapsulated aqueous phase, and this may be responsible for the low yield of nylon 6.10 microcapsules obtained. It was also observed that what appeared

to be small particles of polymer were present in the polyphthalamide microcapsule suspensions. These were not found in the suspensions of nylon 6.10 microcapsules and are thought to have arisen as a result of polymerisation in the bulk organic phase caused by the increase in diacid chloride concentration. This is similar to the formation of the amorphous mass formed in the nylon 6.10 system in the presence of increased sebacoyl chloride. Alternatively, these particles may arise due to the break up of distorted emulsion droplets as illustrated in Figure 4.2 (A3, C5 and D5). If sufficient diamine is contained within these small droplets, it is expected that polymerisation would occur. It might also be expected however, that such particles would form in the nylon 6.10 system. In addition, these particles may arise as a result of impurities present in the phthaloyl chloride which unlike sebacoyl chloride does not require frequent purification to effect microcapsule formation. This difference between the two microencapsulation systems may be due to an increased tolerance toward the hydrolysis products of phthaloyl chloride as compared with those of sebacoyl chloride. Alternatively, it may be due to a difference in the concentration of hydrolysis products. Impurities present in the phthaloyl chloride which do not appear to affect the formation of microcapsules may nevertheless promote polymerisation in the organic phase resulting in the formation of particles. Such particles however, were not observed in nylon 6.10 microcapsule suspensions prepared using impure sebacoyl chloride of sufficient purity to effect capsule formation.

There have been few reports in the literature concerning the structure of polyamide microcapsules although it is usually assumed that they consist of a hollow core surrounded by a thin wall of polymer.

Exceptions to this are the microcapsules prepared by Jenkins and Florence (106) which had the appearance of solid particles. Similarly nylon microcapsules containing a solid core were prepared by McGinity and others (90). The hollow nature of both the nylon 6.10 and polyphthalamide microcapsules prepared in this study was clearly demonstrated using scanning electron microscopy techniques. Figures 3.2 and 3.4 for example show micrographs of suspensions of freeze fractured microcapsules in which the interiors of the microcapsules are visible and which suggest that the microcapsule walls are thin relative to the diameter. Several authors have estimated the thickness of microcapsule membranes prepared by interfacial polymerisation. The thicknesses reported have varied widely according to the polymerisation conditions used. On the basis of electron microscope observations, for example, Chang and others (29) reported that the membranes of their nylon 6.10 haemoglobin containing microcapsules were about 0.02μm in thickness. Lim and Moss (111) however, estimated the membrane thickness of polyamide microcapsules prepared by their method to be approximately 0.08μm. Transmission electron micrographs of the microcapsules prepared in this study showed that the walls of both the nylon 6.10 and polyphthalamide microcapsules were approximately 0.7μm in thickness. This is considerably greater than the values reported by other workers (29, 95, 100, 111). This disparity is believed to be due to differences in the methods of microcapsule preparation, in particular the presence of crosslinking agents. The use of different crosslinking agents in the preparation of microcapsules with modified walls in this study however, resulted in little difference in microcapsule wall thickness (Section 3.7).

In conclusion, microcapsule properties vary widely according to the microencapsulation conditions used. Important parameters in determining the properties of microcapsules prepared by the interfacial polymerisation technique appear to be stirring speed, surfactant concentration, the presence of crosslinking agents, monomer type and monomer concentration. The microcapsules formed by the methods developed in this investigation were found to be hollow mainly spherical particles with diameters in the 5 to 60μm size range. The mean volume diameters of nylon 6.10 and polyphthalamide microcapsules were 16.0μm and 26.0μm respectively. The wall thickness in both cases was estimated to be 0.7μm.

4.2 Microencapsulation of Pilocarpine Nitrate

The preparation of microcapsules containing drug molecules generally involves incorporation of the drug into the microcapsule core material prior to formation of the microcapsule wall. Alternatively, the drug substance may be the total microcapsule core about which the wall is formed. For example, in the preparation of sulphamethoxazole microcapsules by the gelatin-acacia coacervation technique, the polymeric wall forms around micronised crystals of sulphamethoxazole (143). Similarly, the physical methods of microencapsulation generally involve deposition of the wall material about solid particles (151) (Section 1.2.2). In the preparation of microcapsules by the interfacial polymerisation technique and some microencapsulation methods involving non-aqueous phase separation techniques, the drug to be encapsulated is initially dissolved or dispersed in a liquid core material which is then emulsified. The wall of the microcapsules is formed around the individual emulsion droplets. For example, in the formation of polyamide microcapsules containing sodium pentobarbital in an aqueous core, a water in oil emulsion was formed prior to the polymerisation of two monomers at the water/oil interface, resulting in the formation of nylon microcapsules containing the drug (99). In the preparation of such emulsions it is predicted that, dependent upon the lipophilicities of the drugs to be encapsulated, the nature of the organic phase and the relative phase volumes present, a given fraction of the drug included in the initial phase will partition to the organic phase. The high surface area of the dispersed phase together with the high agitation rate will contribute to the speed at which this partitioning occurs. The fraction of drug present in the organic phase will remain unencapsulated. In the microencapsulation of

drugs in polyamide microcapsules containing various matrices, McGinity and others (90) reported that approximately 50% of the diphenhydramine hydrochloride to be encapsulated was lost to the organic phase during the microencapsulation process. The partition of sulphathiazole sodium, sodium salicylate, phenytoin,diazepam and theobromine during the preparation of these microcapsules was however considerably less. Similarly, Naik (123) reported that during the encapsulation of a number of sulphonamide derivatives in polyamide microcapsules, 88 to 96% of the solute included was encapsulated. In this investigation the nitrate salt of pilocarpine was selected for study as this is the least lipophilic of the pilocarpine salts (152) and is therefore expected to yield the highest concentration of pilocarpine in the encapsulated phase. In the absence of polymerisation however, approximately 50% of the pilocarpine nitrate initially included in the microencapsulation system partitioned into the organic phase (Table 3.12). This loss is considered to arise as a result of the relatively large volume of organic phase used in the microencapsulation system and the presence of chloroform in which pilocarpine nitrate is slightly soluble. A similar percentage was lost to the organic phase when polymerisation was allowed to occur and this fraction was found to be independent of concentration (Tables 3.10 to 3.12). The 50% initial pilocarpine nitrate mass remaining in the aqueous microcapsule core was found to be readily removed by the acetone and Tween 20 solutions used to wash and resuspend the formed drug containing microcapsules (Tables 3.10 and 3.11). This further loss of pilocarpine nitrate from the microcapsules was prevented by harvesting the microcapsules directly from the cyclohexane wash in which the drug is poorly soluble (152). In this way approximately half the initial

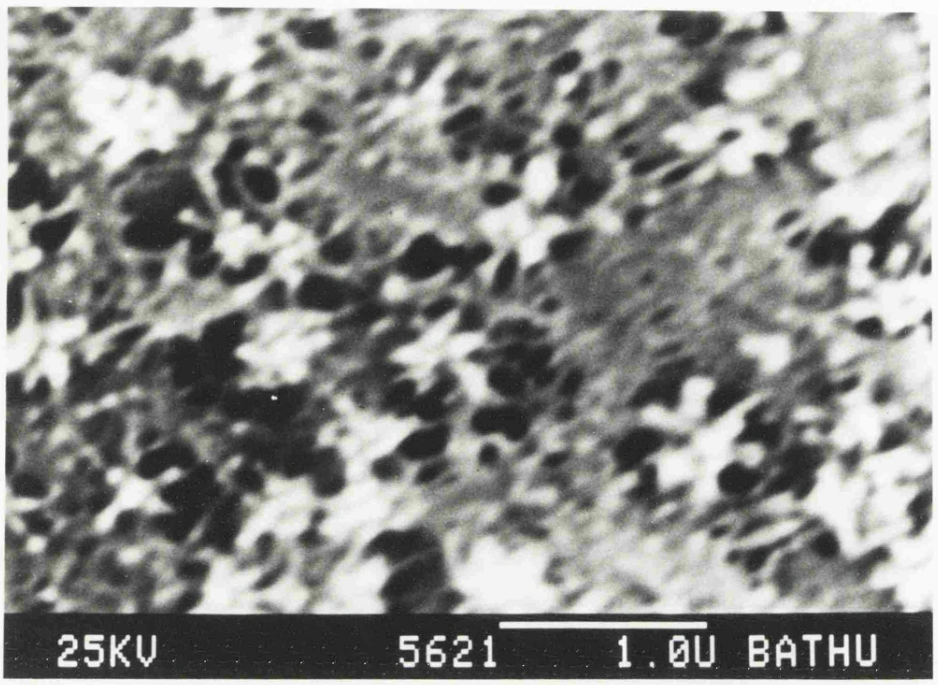
mass of drug included in the preparation remained associated with the microcapsules. This value, which was again found to be independent of concentration, is consistent with the total mass of pilocarpine nitrate recovered from microcapsule suspensions during release studies (Tables 3.17 - 3.21).

Another factor which may influence the incorporation of a drug into microcapsules prepared by an interfacial polymerisation method is the interference of the drug with the polymerisation reaction. This phenomenon has been reported in the encapsulation of aniline (54) and of the quarternary ammonium compounds methantheline bromide and benzalkonium chloride (90). Reaction is thought to occur between nitrogen containing groups present in the drug molecules and the acid chloride functions thereby terminating polymerisation. It is possible that this may also occur in the encapsulation of pilocarpine nitrate as no microcapsules were formed when the drug was included in the internal aqueous phase of the microcapsule system at a concentration of 18.5×10^{-2}M and above (Table 3.13). Pilocarpine nitrate may also interfere with the formation of the polymer at lower concentrations as microcapsule suspensions prepared containing 14.78×10^{-2}M pilocarpine nitrate contained a large number of agglomerates. Supportive evidence of this interaction however, was not found from scanning electron micrographs of the surface of the microcapsules prepared in the presence of 1.85×10^{-2}M pilocarpine nitrate (Figure 4.3). The upper micrograph shows what appear to be star shaped crystals present in surface discontinuities. These crystals are thought to be pilocarpine nitrate, buffer salts or unreacted monomer which has migrated to the surface during drying. However, the surface

appearance of the same sample of microcapsules after washing with water prior to preparation for electron microscopy (Figure 4.3b) is similar to that of microcapsules formed in the absence of pilocarpine nitrate (Figure 3.27). HPLC analysis of pilocarpine nitrate released from the microcapsules failed to detect the presence of any degradation products (Figure 3.15) which indicates that the microencapsulation technique had no adverse affect on the stability of the pilocarpine nitrate molecule.

An alternative method by which drugs might be incorporated into the core of microcapsules with permeable walls involves immersing formed microcapsules, prepared in the absence of the drug, in a solution of the drug until equilibrium between the microcapsule core and the solution is reached. By this method there can be no interference of the drug with the polymerisation reaction. In the preparation of polyphthalamide microcapsules containing pilocarpine nitrate this was achieved by immersing freeze dried polyphthalamide microcapsules prepared in the absence of pilocarpine nitrate, in an aqueous solution of the drug for 12 hours. Determination of pilocarpine nitrate uptake onto the whole and broken freeze dried microcapsule walls (Table 3.16) together with observations of wall water permeability suggest that the microcapsule walls were permeable to pilocarpine nitrate and that equilibration between the microcapsule core and the solution occurred rapidly.

Thus there are two methods by which drugs may be included in the aqueous core of microcapsules prepared by the interfacial polymerisation technique. Inclusion of compounds containing nitrogen prior to polymerisation however, may interfere with the polymerisation reaction.



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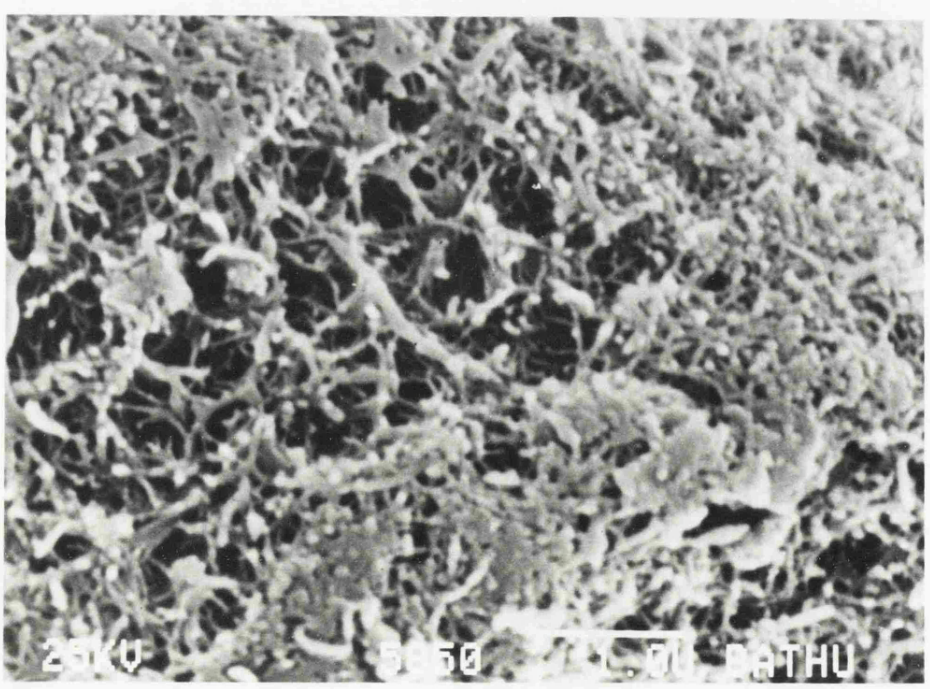


Scale: ⊢——⊣
1.0μm

FIGURE 4.3 SCANNING ELECTRON MICROGRAPHS OF THE SURFACE OF POLYPHTHALAMIDE MICROCAPSULES a) CONTAINING PILOCARPINE NITRATE, b) AFTER REMOVAL OF PILOCARPINE NITRATE.

4.3 Release Characteristics of Polyphthalamide Microcapsules

Two possible mechanisms have been proposed for the release of drugs from microcapsules; disintegration of the capsule or diffusion of the drug through the microcapsule wall. In most cases with the exception of biodegradable microcapsules, drug is released via diffusion through the wall which remains intact. The rate of release of drugs from microcapsules and microcapsule formulations has been the subject of several investigations. For example, materials whose release from coacervate walled microcapsules has been studied include thiabendazole, various barbiturates and sulphamethoxazole (145, 153, 154). Similarly the release of isoniazid, phenobarbitone sodium and salicylamide from cellulose walled microcapsules has been investigated (50, 142, 155).
In particular, the rate of release of drugs from polyamide microcapsules prepared by the interfacial polymerisation technique has been studied by several workers (99, 118, 119, 122) and the reported methods for determining the release profiles have varied widely. Prior to release determination McGinity (107) air dried sulphathiazole sodium and gelatin containing polyamide microcapsules to remove formalin, organic solvents and water. The release of the drug into 0.1M HCl and 0.1M acetate buffer in a round bottom flask maintained at 37^{o}C was then studied. The dissolution medium was stirred at 100rpm and the release of the drug was followed spectrophotometrically. In contrast, Luzzi and others (99), either spray dried or flash evaporated microcapsule suspensions containing sodium pentobarbital prior to release measurements. The release was again determined at 37^{o}C, but the dissolution media in this case were 0.1M HCl, 0.1M KH_2PO_4 - KOH buffer pH 6.75 or distilled water and the stirring speed was only 6 rpm.

Alternatively, the method used by Florence and Jenkins (122) for the determination of release of trifluoperazine embonate from nylon 6.10 microcapsules involved placing a quantity of microcapsule suspension in a Visking bag. In this case the release into pH 7.4 buffer was determined. The buffer was stirred at 60rpm. From these investigations it is apparent that the rate of release of drugs from microcapsules is a function not only of the method used to prepare the microcapsules and the nature of the encapsulated solute, but also of the method by which release is determined. In particular, it is dependent upon the nature of the dissolution medium and it is expected that it will also be dependent upon agitation rate. In this study, four in vitro methods for the determination of the release of pilocarpine nitrate from polyphthalamide microcapsules were developed. Firstly, following polymerisation, pilocarpine nitrate containing microcapsules were removed from the cyclohexane wash and rotary evaporated to remove the residual organic solvent prior to immersion in the release medium. Secondly, polyphthalamide microcapsules prepared in the absence of pilocarpine nitrate were freeze dried. The microcapsules were then immersed in a solution of pilocarpine nitrate until equilibrium was reached, following which the release from the suspension was determined. The third method used involved freeze drying of suspensions of microcapsules prepared in the presence of pilocarpine nitrate prior to the determination of release, and the fourth method consisted of partial freeze drying of microcapsule suspensions. This was achieved by drying for a period of only 30 minutes. In all cases the powder or suspension obtained was dispersed in 50ml isotonic phosphate buffer, pH 7.4 at $32^{o}C$; these values are approximately the pH of tear fluid and the

temperature of the cornea respectively. Analysis of pilocarpine nitrate was carried out using liquid scintillation counting techniques with one exception where HPLC was used. This was undertaken to ensure that the release profiles obtained were those of pilocarpine nitrate and not of either a labelled degradation product or a tritium exchange product (Figure 3.14). Furthermore, observations of microcapsule appearance (Figure 3.9) and size distribution (Figure 3.10) following freeze drying of microcapsule suspensions indicate that this method of harvesting has no detrimental effect on the microcapsules which were found to collapse on drying and to reconstitute well on dispersion in water. It was observed however, that the powder was very hygroscopic, absorbing moisture from the atmosphere immediately after removal from the freeze drier. This is thought to give rise to the large variation in concentration observed in samples removed from different batches of freeze dried material which may be seen in Table 3.15. The release profiles obtained using each of the four methods of release described above are given in Figures 3.11 to 3.14. Inspection of the data (Tables 3.17 to 3.21) indicates that in all cases the release of pilocarpine nitrate was rapid. There are however, differences between the profiles obtained for the release from the microcapsules determined by the different methods containing the same initial pilocarpine nitrate concentration. For example, the release profiles from microcapsules initially containing 1.9×10^{-2}M pilocarpine nitrate in Figures 3.11 and 3.12 suggest that the initial release from the microcapsules is faster when determined by Method 2 as compared with Method 1. Using Method 1, after 5 minutes approximately 78% of the total mass of pilocarpine nitrate released at equilibrium was lost whereas for Method 2 the value was 97%.

Similarly the initial release rate is higher in Method 2 than in Methods 3 and 4. There are a number of factors which may be responsible for these differences such as the presence of trace amounts of solvent or the presence of unencapsulated drug. Using Methods 1 and 4, following rotary evaporation or partial freeze drying, trace amounts of cyclohexane may remain associated with the microcapsule walls which will prevent the aqueous medium from wetting the microcapsule surface. Conversely, over drying the microcapsule surface during freeze drying may result in an apparent delay in the release due to a finite time requirement to re-wet the surface. Differences between the profiles obtained using Method 2 and Methods 1, 3 and 4 however, may simply be due to masking of the initial true release rate in Method 2 brought about by the rapid dilution of the extra capsular pilocarpine nitrate present in solution. Similarly, adsorption of moisture from the freeze dried microcapsules may produce an initially elevated release rate due to the adsorbed water prematurely leaching drug from the core. Elevated release rates will also be observed if solute migration, such as that which takes place during the drying of granules (156), occurs during drying of microcapsules. That is, as the surface liquid evaporates, so it is replenished with water from the core carrying with it dissolved solute; this water then evaporates leaving the solute on the surface of the microcapsules. This phenomenon may also occur during flash evaporation, spray drying and air drying (99, 107). Artificially high release rates will also be observed if, as is indicated by the yields obtained (Table 3.8) incomplete encapsulation of the aqueous phase takes place during polymerisation. The unencapsulated pilocarpine nitrate solution in this case is thought to collect at the microcapsule surface

during centrifugation. It is apparent therefore that, due to the physical nature of the polyamide microcapsules and the methods by which they are prepared for release studies, comparison of release data obtained using different methods of release determination cannot be made. Of the methods described above partial freeze drying was considered to be the method of choice as this was the only method by which gelatin containing microcapsules could be successfully dried and reconstituted (Section 3.6.1).

Some variation in the release profiles obtained from two batches of microcapsules containing the same initial drug concentrations in which the release is determined by the same method may also be observed in Figures 3.11, 3.12 and 3.14. In the release from microcapsules prepared containing 3.61×10^{-2}M pilocarpine nitrate determined by Method 1 (Figure 3.11), after 120 minutes 3.017×10^{-3} moles of drug had been released per gram of microcapsule slurry for determination 1, which compares with a value of 2.532×10^{-3} moles in the case of determination 2. Similarly in the release of drug from microcapsules initially containing 1.9×10^{-2}M pilocarpine nitrate determined by Method 4 after 120 minutes the total mass released per gram of microcapsule slurry was found to be 1.678×10^{-5} moles (determination 1) and 1.534×10^{-5} moles (determination 2). This is to be expected however due to the batch to batch variation in the 'concentration' of microcapsules in the slurry produced after rotary evaporation and freeze drying (Tables 3.8 and 3.15) together with the variation in the distribution of pilocarpine nitrate during the microencapsulation process (Table 3.11). It is also not surprising that the greatest differences are seen in the release profiles obtained for freeze dried material (Figure 3.13) as this product yields the greatest variation in concentration (Table 3.15).

The effect of pilocarpine nitrate concentration on the release rate from polyphthalamide microcapsules is also illustrated in Figures 3.11 to 3.13. There have been few reports in the literature concerning the effect of encapsulated solute concentration on the permeability of microcapsule walls and microcapsule release characteristics, although it was noted by Naik (123) that high solute concentrations interfered with the microencapsulation process and influenced the rate of release. The drug release profiles of microcapsules prepared containing 1.85×10^{-2}M, 3.61×10^{-2}M, 7.36×10^{-2}M and 14.78×10^{-2}M pilocarpine nitrate in Figure 3.11 (Tables 3.17 and 3.18) show that at all concentrations the initial release of pilocarpine nitrate was rapid. At initial pilocarpine nitrate concentrations of 1.85×10^{-2}M and 3.61×10^{-2}M however, a plateau in the release profile was reached within 60 minutes and no further release of the drug was observed up to 120 minutes. At the higher concentrations some reduction in the release rate occurred following the initial rapid release although a plateau was not attained. Consideration of the distribution of the drug during preparation however, (Table 3.18), suggests that at these higher drug concentrations no appreciable amount of pilocarpine nitrate remained associated with the microcapsules after 120 minutes. For example, from one batch of microcapsules prepared containing 14.78×10^{-2}M pilocarpine nitrate, 5.65×10^{-5} moles were released within the first 5 minutes and following this a further 3.357×10^{-5} moles were released over a 115 minute period. In total therefore, 9.007×10^{-5} moles were released representing 55.6% of the amount included, 48.3% already being lost to the organic phase during preparation. Similarly, the drug release profiles of microcapsules prepared containing approximately 1.8×10^{-2}M and 14.8×10^{-2}M pilocarpine nitrate given in Figure 3.13

and Table 3.20 show that at both concentrations the initial release of drug was rapid. At the lower pilocarpine nitrate concentration a plateau in the release profile was reached within approximately 10 minutes, after which time no more pilocarpine nitrate was released. A plateau which corresponds to about 20×10^{-5} moles pilocarpine nitrate was not reached in the release from the microcapsules containing the higher concentration of drug until approximately 30 minutes. In contrast however, Figure 3.12 (Method 2) indicates that for initial pilocarpine nitrate concentrations of 1.8×10^{-2}M and 14.8×10^{-2}M a plateau in the release profile was reached in both cases in 5 minutes. The plateaux corresponded to approximately 6×10^{-5} moles per gram and 50×10^{-5} moles per gram for the lower and higher concentrations respectively. This suggests that the concentration of encapsulated drug does not affect the release from the microcapsules. Rather, it is the concentration of solute present during polymerisation which affects the permeability of the microcapsule walls and therefore their release properties. This is further evidence that pilocarpine nitrate interferes in the polymerisation reaction (see Section 4.2) and is in agreement with the work described by Naik (123).

As discussed in the preceding paragraphs under all conditions studied the release of pilocarpine nitrate from polyphthalamide microcapsules was extremely rapid. From several reports in the literature on the permeability of microcapsules to electrolytes and other 'small' molecules (95, 98, 100, 111, 118, 119) this type of release is to be expected. Chang and Poznansky for example (119) reported the equilibration time for sucrose permeating into polyamide microcapsules as 35.5 seconds and for urea the value was 4.3 seconds. Similar values were reported for the permeation of glycerol, glucose

and sucrose through the walls of poly (hexanediamine terephthaloyl chloride) microcapsules (111). In terms of release profiles Luzzi and others (99) found that the time taken for the release of 50% of the sodium pentobarbital content of nylon 6.10 microcapsules was in the order of 10 to 15 minutes whereas Degennaro (108) reported that 80 to 90% of the total solute content of polyamide microcapsules prepared by their method was released within the first 5 minutes in most cases. This contrasts to the findings of Florence and Jenkins (122) who reported release of trifluoperazine embonate from nylon 6.10 microcapsules over periods of up to 64 hours. In this study 50% of the total mass of pilocarpine nitrate released from the polyphthalamide microcapsules at equilibrium was lost within 2 minutes independent of the method of release measurement used. With the exception of the studies by Degennaro (108) this is considerably faster than the release rates reported by other authors and may be due to the methods of preparation and release determination used. For comparison of the release data obtained Table 4.1 gives the $t_{50\%}$, $t_{90\%}$ and M_{10} values for each of the systems investigated. (Where $t_{50\%}$ and $t_{90\%}$ are the times taken to release 50% and 90% of the total mass of pilocarpine nitrate released at equilibrium and M_{10} is the mass released in 10 minutes expressed as a percentage of the total mass released at equilibrium.) In all cases 90% of the total amount released was lost within 20 minutes and 50% within one minute for an initial pilocarpine nitrate concentration of 1.85×10^{-2}M. The M_{10} values are in the range 82 to 100%. For an initial pilocarpine nitrate concentration of approximately 14.7×10^{-2}M, 50% of the total mass of pilocarpine nitrate released was lost within 2 minutes and the M_{10} values range between 66% and 100%. These findings suggest that the microcapsule walls are

Solute	Pilocarpine Nitrate.									Prednisolone Sodium Phosphate.
Release Method	1				2		3		4	4
Concentration of Solute in Initial Aqueous Phase. x 10^2M	1.8	3.7	7.4	14.8	1.8	14.8	1.8	14.8	1.8	1.8
$t_{50\%}$ (minutes)	1	1	1	2	1	1	1	1	1	1
$t_{90\%}$ (minutes)	20	20	65	60	1	1	4	15	1	20
M_{10} (%)	82	76	71	64	100	100	99	88	96	86

TABLE 4.1 RELEASE OF PILOCARPINE NITRATE AND PREDNISOLONE SODIUM PHOSPHATE FROM POLYPHTHALAMIDE MICROCAPSULES.

Where: $t_{50\%}$ and $t_{90\%}$ are the appr oximate times taken in minutes to release 50% and 90% respectively of the total solute content released at equilibrium.

M_{10} is the mass of solute released in 10 minutes expressed as a percentage of the mass released at equilibrium.

ineffective in significantly controlling the release of pilocarpine nitrate and indicate that the walls are freely permeable to the drug.

In order to ensure that this rapid release rate is not peculiar to the pilocarpine nitrate molecule, the release from microcapsules prepared containing prednisolone sodium phosphate was investigated. This compound was selected as it is of a similar size to pilocarpine nitrate, is readily assayed and contains no reactive nitrogen groups. The release of the prednisolone sodium phosphate was again rapid (Figure 3.16). The $t_{50\%}$, $t_{90\%}$ and M_{10} values are given in Table 4.1 from which it may be seen that the release was only slightly slower than that obtained for pilocarpine nitrate using the same method of release measurement. It has been suggested that the permeability of polyamide microcapsule membranes to various solutes is a function of their molecular weight (111, 119); for example, Lim and Moss reported the half equilibration times for the permeation of glycerol (moleculon weight 92) glucose (molecular weight 180) and sucrose (molecular weight 342) through the walls of polyamide microcapsules as 1.8, 4.9 and 10.6 seconds respectively. The difference in release rates observed for prednisolone sodium phosphate and pilocarpine nitrate may therefore be a function of the molecular size of the two solutes. (The molecular weight of prednisolone sodium phosphate is 484 and that of pilocarpine nitrate is 271). This dependency of release rate upon molecular size may also give rise to the widely different release rates reported by authors working with different solutes.

It is considered that the rapid release of both pilocarpine nitrate and prednisolone sodium phosphate from the polyamide microcapsules may also be a function of the large surface area to volume ratio of the microcapsules together with the relatively large volume of release medium resulting in sink conditions. That is the surface area of 4.0ml microcapsules with a mean volume diameter of 26μm is calculated to be approximately 9,200 cm^2. In vitro release from these 4.0ml microcapsules was determined by dispersion into 50ml buffer solution. These conditions are considerably different from the in vivo situation in the eye in which it was considered the microcapsules may exhibit some sustained release effect. The in vivo data, however, similarly suggest that the release of pilocarpine nitrate from the polyphthalamide microcapsules is rapid. That is, there was no difference in the miotic response induced by encapsulated pilocarpine nitrate as compared with pilocarpine nitrate solution following instillation into the rabbit eye (Figure 3.40). In both cases the pupil diameter reached a minimum at 30 minutes post instillation and returned to its initial size after approximately 180 minutes. Miosis was used as an indication of pilocarpine kinetics (17, 157, 158) as it was found that there is no intraocular pressure response to pilocarpine in the rabbit eye. The in vivo release data given in Figure 3.40 may indicate either that the microcapsules have no significant effect in controlling the release of pilocarpine nitrate or that the microcapsules are washed immediately from the eye and therefore do not prolong the contact time of the drug. This second hypothesis is not consistent with the study of microcapsule dwell time in the eye. The dwell time of microcapsules containing gamma labelled albumin which is known to remain associated with the microcapsules (Section 4.4) was investigated using a gamma

camera technique. 88% of the activity associated with the microcapsules instilled in the rabbit eye remained in the eye after one minute and after twenty minutes this value had decreased by only a further 3% (Figure 3.41). In comparison, on instillation of a gamma labelled albumin solution the activity decreased to 39% within only one minute. From this, it may be concluded that the microcapsules are effective in significantly increasing dwell time in the eye when compared with the albumin solution and also with viscous eye drops (15). The microcapsules however, do not appear to control the *in vitro* or *in vivo* release of encapsulated pilocarpine nitrate or the *in vitro* release of prednisolone sodium phosphate.

4.4 Mechanism of Release of Pilocarpine Nitrate from Polyphthalamide Microcapsules

The rapid release of both pilocarpine nitrate and prednisolone sodium phosphate from polyphthalamide microcapsules discussed above, suggests that the polyamide microcapsule walls are freely permeable to small solutes. The nature of the interaction mechanism between small solutes, in particular weak organic acids and bases, and polyamides has been the subject of several reports in the literature. Richardson (67) showed that the sorption of a weak base, ethyl-4-aminobenzoate, by nylon 6 powder involves penetration of the drug into the polymer matrix. The mechanism of the interaction is unclear but is believed to involve hydrogen bond formation between the drug and the amide groups in the polymer chain. The bonds may then be stabilised by Van der Waals' forces. Similarly Kapadia and colleagues (72) investigated the interaction of a number of 4-hydroxybenzoate derivatives with polyamides and proposed that these solutes formed very weak hydrogen bonds with the amido groups in the polymer which were then stabilised by Van der Waals' forces. In contrast, other workers have suggested that the interaction between polyamides and organic acids or chlorbutol involves forces only of the Van der Waals' type (70). A close correlation between the extent of the interaction and the percentage of solute in the unionised form has been found by several workers. Richardson (67) reported on the sorption of ethyl-4-aminobenzoate by nylon 6 powder and concluded that when the drug was present in the ionised form there was no detectable sorption. Similar findings were reported by Ho (68) for the sorption of ethyl-4-aminobenzoate by nylon 6 films and by Kapadia and others (72) for the sorption of salicylic acid by nylon 6.6. The state of ionisation of weak aromatic acids and bases has also been shown to influence their permeation through polyamide films. For ethyl-4-

aminobenzoate permeation in which determinations were made using a simple permeation cell, Ho (68) observed that in the completely ionised state the drug did not permeate commercially prepared nylon 6 films. In the unionised form however, permeation did occur. It is unlikely therefore that a charged molecule which is not capable of forming a specific interaction with polyamides would permeate through nylon films. Figure 3.39 shows that pilocarpine nitrate in pH 7.4 buffer and ionised ethyl-4-aminobenzoate do not permeate commercially prepared nylon 6 films which is in agreement with these findings. Similarly, ethyl-4-aminobenzoate in the unionised form was found to permeate the nylon 6 film (Figure 3.39). The permeability coefficient of unionised ethyl-4-aminobenzoate through the nylon 6 film was calculated to be approximately 1.1×10^{-12} M^2 sec^{-1} with a lag time of about 40 minutes, which is in good agreement with values reported by other workers (68, 73). This lag time is believed to be due to the time taken for the drug to penetrate the polymer matrix at the donor side and diffuse to the receptor side. The permeability data for pilocarpine nitrate and ionised and unionised ethyl-4-aminobenzoate through polyphthalamide films prepared by interfacial polymerisation (Figures 3.37 x 3.38) however, is inconsistent with accepted drug-nylon interaction theory. In all cases, equilibrium between the receptor and donor solutions in the permeability cells was achieved within 120 minutes and no measurable lag time was observed. The absence of a lag time suggests that the solutes are not diffusing through the polymer matrix solely at a molecular level and may indicate the presence of water filled pores or channels in the film. Figure 3.38 also shows that there was no apparent difference in the permeation rate of ionised and unionised ethyl-4-amino-benzoate again suggesting that the polyphthalamide films are different in nature from commercially

prepared nylon 6 films. The absence of a linear portion of the permeability profiles together with the variability in film thickness make calculations of permeability coefficients through the polyphthalamide films difficult. Estimations of the permeability over the time period 5 to 30 minutes however, give values of 4.7×10^{-11}, 7.1×10^{-10}, 6.3×10^{-10} $M^2 sec^{-1}$ for pilocarpine nitrate, ionised and unionised ethyl-4-aminobenzoate respectively. These are approximately 500 times greater than that for unionised ethyl-4-aminobenzoate through the commercially prepared nylon 6 film (Figure 3.39), and are consistent with permeation occurring not by diffusion through the polymer matrix but by another mechanism such as via water filled channels.

Extrapolation of the data obtained for the polyphthalamide films to the microcapsule walls must however be treated with caution. Although the films used were prepared by an interfacial polymerisation method based on that used in the preparation of microcapsules, modifications to this method were necessary. In particular, changes to the monomer concentration and solvent composition were made in order to produce films of sufficient strength to be drawn from the interface. Also, the support of a grid was necessary to maintain the integrity of the film in the diffusion cell (Section 3.8.4). In addition, it was not possible to prepare films of large diameter and this therefore necessitated using small aperture diffusion cells. As a result of corresponding small volume of the receptor compartment in these diffusion cells, volumes of solution removed for assay were significant and removal of samples resulted in a differential hydrostatic pressure across the film. Furthermore, the fragility of the films prevented the donor and receptor solutions being stirred at

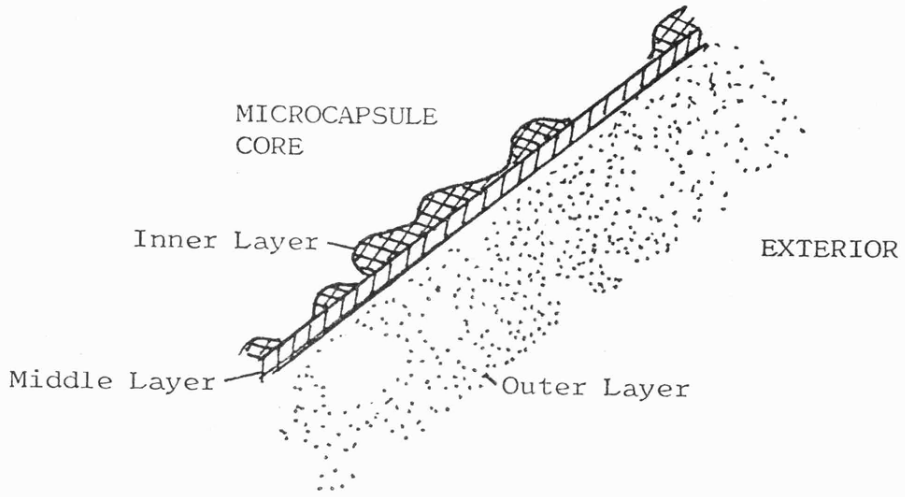
speeds greater than 300 rpm. In contrast to the microcapsule system however, the films were able to limit the permeation of the pilocarpine nitrate. This may be due to the relative film/wall thicknesses and the surface area. Film thicknesses were estimated to be in the order of 70μm with a surface area of 9.62 cm^2 and a donor compartment volume of 50ml. The microcapsules, in comparison, had a wall thickness of about 0.7μm and for a capsule volume of 4ml the calculated surface area is over 9,000 cm^2.

It is apparent from the above discussion that a mechanism other than that involving diffusion of solute through the polymer matrix governs the release of drugs from polyphthalamide microcapsules. The release mechanism is thought to be determined by the structure and physical properties of the microcapsule walls. The appearance of polyamide membranes prepared by interfacial polymerisation has been described by both Morgan and Kwolek (81) and Enkelmann and Wagner (79). The surface of the film adjacent to the aqueous phase was found to be smooth, whereas that adjacent to the organic phase was rough. This type of structure is also observed in the scanning electron micrographs of freeze fractured nylon 6.10 and polyphthalamide microcapsules (Figures 3.3, 3.4 and 3.9). The inside of the walls are smooth whereas the outer surface of the walls are rough and suggest the presence of pores or surface discontinuities. The surface of the microcapsules prepared by McGinity and others (90) were also found to be rough and those prepared containing a gelatin matrix were reported to have a rough and porous surface with a plate-like structure. In contrast, the outer walls of some pesticide containing microcapsules appear smooth (34). Again this is in agreement with the findings of Enkelmann and Wegner (79)

and Morgan and Kwolek (81) as in this case the polymer is formed in an emulsion where the continuous phase is aqueous and the dispersed phase is an oil. The surface appearance of microcapsules prepared by interfacial polymerisation was also reported by Jenkins and Florence (106) who concluded that the walls were highly porous in nature. The pores were considered to be of a filamentous structure and those seen in films prepared by interfacial polymerisation ran from one surface of the film to the other which, it was concluded, agreed with the radiating structure of polymer strands in the capsules. It is also consistent with the findings of Enkelmann and Wegner (79) who suggest that in interfacial polymerisation the polymer chains form perpendicular to the surface. These structures in all cases differ from the nylon 6 powder described by Wicks (73) which was considered to be highly crystalline in nature and non-porous, although the surface was highly convoluted. This suggests that the properties of polyamides prepared by interfacial polymerisation may generally be expected to differ from those prepared by more conventional techniques, which is consistent with the permeability data discussed above.

Using transmission electron microscopy, investigation of the pores present in the microcapsule walls prepared in this study again indicated that the surface was porous in nature but did not confirm that any of these pores penetrated to the centre of the core. The micrographs in Figure 3.28 show that the walls are composed of 3 distinct layers which are shown diagrammatically in Figure 4.4.

Figure 4.4 The Structure of Polyphthalamide Microcapsule Walls



The three layers from the core outwards are:

1. A dense discontinuous nodular layer. This is thought to be formed due to the presence of polyethyleneimine which would initially form a dense crosslinked polymer network at the interface as discussed in Section 4.1. As polymerisation proceeds the diffusion of the polyethyleneimine will be limited preventing further crosslinking in the outer layers of the wall (see Figure 4.1).

2. A narrow dense continuous layer. This layer is probably the site of maximum polymerisation between the diamine and the diacid chloride

3. A relatively thick loosely attached less dense layer.

This type of structure is consistent with the report that as the membrane thickness increases the structure of the membrane becomes more and more disordered and the outer layers are composed of relatively low molecular weight polymer (79). As polymerisation reaches completion several factors may give rise to the formation of this lower molecular weight polymer. These include hydrolysis of the diacid chloride and depletion of the diamine in the polymerisation zone brought about by its reduced rate of diffusion through the already formed membrane. Such low molecular weight material may result in the loose 'amorphous' layer observed in the microcapsule walls. It is this outer layer which is considered responsible for the surface appearance of the microcapsules suggesting the presence of pores or discontinuities.

Pores and voids may arise as a result of the method by which the microcapsules are prepared. As polymer is formed on the organic side of the interface solvent may be trapped during polymerisation. Polyamides have a low affinity for organic solvents and on drying it might be expected that this will be removed resulting in pores and channels. Other workers have previously reported the presence of pores in microcapsule wall materials. Okohata and others (159) clearly showed the presence of pores of approximately 1μm diameter in the surface of polyamide microcapsules of approximately 2.5mm diameter. Transmission electron micrographs indicated that these pores penetrated the wall. Using measurements of the effective osmotic pressure of a number of electrolytes across the membranes of their nylon 6.10 microcapsules (of approximately 270μm diameter), Chang and others (29) estimated the size of pores present to be about 1.6nm. By increasing the reaction time during preparation

from 3 to 15 minutes this pore size could be reduced to 0.5nm (30). This reduction in pore size may be due to more extensive polymerisation within the microcapsule membrane giving rise to a more dense polymer matrix. A conflicting finding was reported by Ndong-Nkoume and others (160) who found no pores present in the walls of polyamide microcapsules prepared in the presence of L-lysine. This discrepancy is thought to be due to either the presence of the L-lysine or the increased concentration of diacid chloride present, 0.063M in comparison with 0.018M reported by Chang and others. The increased monomer concentration may again lead to the formation of a more dense membrane. Alternatively, the discrepancy may be due to the difference in microcapsule diameter (15μm as compared with 270μm) which it has been reported gives rise to microcapsules with a reduced porosity and hence permeability (161). Pores have also been observed in the walls of microcapsules prepared by other techniques, such as phase separation (162, 163) where it has been suggested that these pores play an important role in determining the release of drugs from the core.

The hypothesis that pores or channels are present in the walls of microcapsules prepared by interfacial polymerisation techniques is further supported by determinations of the molecular weight cut off of the membranes. Miyawaki and colleagues (118) determined the molecular weight cut off of haemoglobin containing nylon microcapsules using a permeability measurement technique. Formed microcapsules were immersed in a solution of tracer (for example salmine, or brilliant blue) until equilibrium was reached. The external solution was then discarded and the release from the microcapsules determined. From this it was found that dextran blue and salmine

were unable to penetrate the capsule wall whereas brilliant blue and phenylalanine permeated the membrane. The molecular weight cut off was therefore reported to lie between 1,000 and 10,000. In contrast, the molecular weight cut off of the membranes of microcapsules prepared in this investigation was found to lie between approximately 20,000 and 40,000. Microcapsules were prepared containing (a) albumin fluorescein isothiocyanate of molecular weight 68,000; (b) fluorescein isothiocyanate dextran of molecular weight 39,000 and (c) fluorescein isothiocyanate dextran of molecular weight 17,500. Following preparation the microcapsules were washed repeatedly with 10ml pH 7.4 isotonic phosphate buffer and the supernatant examined for colour. The supernatant from the microcapsules prepared containing fluorescein isothiocyanate dextran of molecular weight 17,500 was found to be coloured, the remaining two being colourless. Thus it was concluded that the microcapsule walls were permeable to the lower molecular weight dextran but not the remaining two solutes giving rise to the reported value for the molecular weight cut off.

The effect of molecular volume on the diffusion and permeation of solutes through a polyamide film was investigated by Rodell and colleagues (164). It was reported that as the molecular size of a series of 4-hydroxybenzoates (namely p hydroxy, methyl p hydroxy, propyl p hydroxy and butyl p hydroxy benzoates) increased the rate of diffusion and permeation in nylon decreased. For a given set of conditions the permeability coefficient increased from 0.93 to 2.66×10^{7} cm^{2} sec^{-1}. It is unlikely therefore that molecules having a molecular weight of 17,500 will permeate a polyamide matrix within a few minutes which again suggests the presence of water filled channels or pores in the capsule walls.

Attempts to determine pore size using nitrogen adsorption techniques were unsuccessful. Using this method it was calculated that the pore size varied between 1.5 and 15nm and the mean pore radius was estimated to be approximately 4nm (see Appendix A.2). Consideration of the molecular weight of dextran unable to permeate the microcapsule walls (that is, molecular weight 39,000) together with measurements of the pore radius of haemoglobin (molecular weight 68,000, mean pore radius 3.3nm (119)) suggests that this value is an overestimate. Similarly, estimation of the molecular size of the fluorescein isothiocyanate dextran molecules using a photon correlation spectrometer, in order to predict pore size, were unsuccessful. However, it is expected that the presence of pores in the microcapsule walls, sufficiently large to allow the permeation of molecules of molecular weight 17,500, will readily allow the permeation of 'smaller' molecules such as pilocarpine nitrate. The rapid rate of release from the polyphthalamide microcapsules seen in the release profiles (Figures 3.11 to 3.14) is therefore considered to be a function of the presence of pores in the microcapsule membranes.

4.5 Modifications to the Release Characteristics and Permeability of Polyphthalamide Microcapsules

There have been several recent reports in the literature concerning modifications to polyamide microcapsule preparation conditions in an attempt to alter the permeability and release characteristics of the microcapsules. The methods used may be broadly classified into two types. (1) the inclusion of a matrix material in the core, present so as to limit the diffusion of the encapsulated drug to the wall prior to release and (2) modifications to the wall structure such as an increase in the density of the polymer or an increase in the wall thickness in order to decrease microcapsule membrane permeability (161) and therefore limit the diffusion of the drug through the microcapsule membrane. For example, McGinity (107) reported the inclusion of formalised gelatin in the preparation of polyamide microcapsules in an attempt to control the release of sulphathiazole sodium from the core. The release from both the nylon microcapsules and the formalin treated nylon gelatin formulation into 0.1N HCl and 0.1M acetate buffer was found to be rapid however and there was little difference in release rate for the two microcapsule types. A later report investigated the use of other matrix materials such as calcium alginate and calcium sulphate (90). Data concerning the release of drugs from these microcapsules was not given. An example of the second type of modification, that is a change in wall structure was reported by Lim and Moss (111). Following preparation polyamide microcapsules were harvested and redispersed in cyclohexane prior to the addition of a further volume of diacid chloride. On immersing the formed capsules in the cyclohexane any diamine remaining in the microcapsule partitions into the cyclohexane. Due to the poor affinity

of the diamine for the cyclohexane however, the partitioning is reduced relative to that seen in the primary polymerisation in which the organic phase consisted of 1 part chloroform and 4 parts cyclohexane. Addition of a further volume of diacid chloride therefore results in polymerisation with any unreacted diamine within the voids of the already formed network giving rise to a denser membrane. Alternatively, increasing the affinity of the diamine for the second organic phase results in the secondary polymerisation occuring on the organic side of the original membrane producing thicker walls (112). In both cases it was purported that these methods could be used to control the upper limit of permeability of the microcapsules and thereby reduce the diffusion of solutes of given molecular weights through the membranes.

In this investigation attempts to alter the release characteristics of polyphthalamide microcapsules (Figures 3.11 to 3.15) involved both techniques. These included the addition of gelatin or albumin to the microcapsule core prior to polymerisation. Gelatin has previously been used to control the release rate of sulphathiazole sodium as described above whereas the albumin is known to be incorporated into the microcapsule walls and it was considered the pilocarpine nitrate may bind to the albumin thereby reducing the rate of release of the drug.

Other techniques which were developed to control the release of pilocarpine nitrate were the use of short chain crosslinking molecules in place of polyethyleneimine and the preparation of double walled microcapsules based on the two methods reported by Lim and Moss (111,112).

Naik (123) reported that the presence of crosslinking agents in interfacial polymerisation, in particular diethylenetriamine and triethylenetetramine, gives rise to microcapsules with membranes of increased porosity. The pores present in the polyphthalamide microcapsule walls may be due to the molecular size of the polyethyleneimine used in the original preparation. The polyethyleneimine may hold apart the polyphthalamide chains and give rise to an open porous network. The use of short chain crosslinking molecules in the preparation of microcapsules was first described by Vandegaer and Wayne (104). It is thought that these give rise to a more closely crosslinked polymer network than that obtained using polyethyleneimine thus resulting in a denser membrane. Transmission electron microscopy however did not confirm that a denser membrane was formed using either the short chain crosslinking molecules or the double polymerisation technique Method A (Section 3.6.4). In the case of the double walled microcapsules prepared by Method B, electron microscopy indicated that a double wall was formed around the aqueous core (Figure 3.35). The inner wall was a trilaminate structure similar in appearance to the membrane observed in the original polyphthalamide microcapsules, whereas the outer layer was a single dense band. As shown in Figures 3.19, 3.20, 3.23 and 3.26 these modifications were unsuccessful in controlling the release of pilocarpine nitrate. Data obtained from these release profiles is given in Table 4.2 from which it is apparent that in all cases 50% of the total mass of pilocarpine nitrate released at equilibrium was lost in the first minute and 90% in the first four minutes. Some deviation is seen in the release from the microcapsules prepared containing gelatin and those prepared using the short chain crosslinking molecules, suggesting that these modifications were successful in altering

Microcapsule Type.	Gelatin.	Crosslinked Gelatin.	Albumin.	Short Chain Crosslinked.	Double Walled. A.	Double Walled B.	Polyphthalamide.
Release Method.	4	4	4	4	4	4	4
Initial Pilocarpine Nitrate Concentration x 10^2M.	1.8	1.8	1.8	1.8	1.8	1.8	1.8
$t_{50\%}$ (minutes)	1	1	1	1	1	1	1
$t_{90\%}$ (minutes)	3	1	1	3	1	1	1
M_{10} (%)	94	100	100	97	96	95	96

TABLE 4.2 RELEASE OF PILOCARPINE NITRATE FROM POLYPHTHALAMIDE MICROCAPSULES WITH MODIFIED CORE AND WALLS.

the diffusion rate of the pilocarpine nitrate. The deviation is however small and in contrast the effect of the modifications on the appearance of the microcapsules is marked. For example, the reduced Span 85 concentration required to form the microcapsules in the absence of polyethyleneimine is thought to be responsible for the increase in the median volume diameter observed for the gelatin containing microcapsules and those prepared using the short chain crosslinking molecules. These had values of 35μm and 36μm respectively (Figures 3.18, 3.22). Similarly the absence of polyethyleneimine from the microcapsules prepared using the short chain crosslinking molecules is believed to give rise to the agglomerates observed in these microcapsule suspensions together with their irregularity in shape (Figure 3.21). This is consistent with the work of Aisina and others (94) who indicated the need for the presence of a polymeric filler material in the aqueous core of the microcapsules in order to maintain their integrity. In contrast, the collapsed microcapsules and aggregates seen in the suspensions of double walled microcapsules prepared by Method B (Figure 3.24) are thought to arise due to the effect of redispersion in the hypertonic solution of polyethyleneimine followed by the secondary polymerisation occuring between individual microcapsules causing them to adhere. The opaque interior of the microcapsules prepared containing gelatin (Figure 3.17) is probably due to the presence of the gelatin in the microcapsule core.

The modifications to the preparative technique described appear to have had no effect on the surface structure of the microcapsules when examined by scanning electron microscopy (Figures 3.31 and 3.33). This technique could not however be used to study the structure of gelatin containing microcapsules as they collapsed and aggregated

during processing. Similarly the modifications to the methods of preparation had little effect on wall thickness which was estimated to be approximately 0.8μm (Figures 3.32, 3.34, 3.35). Using transmission electron microscopy however it was found that the structure of the walls was dependent upon the method of preparation. The walls of the microcapsules prepared using the short chain crosslinking molecules consisted of only two layers (Figure 3.32) (1) a dense discontinuous outer layer and (2) a thin less dense continuous inner layer. This contrasts with the walls of the polyphthalamide microcapsules which were of a trilaminate structure (see Figure 4.4) and may suggest that the inner dense nodular layer present in the polyphthalamide microcapsules is due to the presence of polyethyleneimine. Similarly, the site of maximum crosslinkage of the polyamide chains and hence maximum membrane density may be a function of the affinity of the crosslinking agents for the different phases. In the case of the short chain crosslinking molecules this may give rise to the formation of the more dense portion of the membrane in the outer layer. The walls of the microcapsules prepared by the double polymerisation Method B were also of a different structure, as discussed above (Figure 3.35). These walls were composed of an inner trilaminate structure comprising the wall of the original polyphthalamide microcapsule and an outer single band formed by the second polymerisation. That this outer layer consists of only a single dense band is thought to be a function of the diffusion rate of the diamine through the already formed microcapsule wall.

It is apparent therefore that it is possible to alter the structure and appearance of polyamide microcapsules considerably by changing the polymerisation conditions. Such modifications as described however, appear to have little effect on the rate of release of small solutes which, under the conditions described was found to be very rapid. Without further modifications the polyphthalamide microcapsules prepared in this investigation are of little benefit in controlling the release of drugs in the eye.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

This study indicates that in the presence of a crosslinking agent, polyethyleneimine, nylon 6.10 and polyphthalamide microcapsules with an aqueous core may be readily prepared by an interfacial polymerisation technique. Electron microscopy revealed that the microcapsules formed using this technique are hollow spheres and have thin walls relative to their diameter. Optimisation of the preparation conditions confirmed reports in the literature that microcapsule size is largely dependent upon emulsification conditions, in particular stirring speed. Microcapsule yield is strongly affected by the concentration and nature of the monomers used.

Small solutes may be incorported in the aqueous core of the microcapsules by two methods; immersion of formed microcapsules in an aqueous solution of the solute or dissolution of the solute in the aqueous phase prior to polymerisation. In the latter method the solute partitions into the organic phase during microcapsule formation and as a result complete encapsulation is not achieved. Furthermore, any reactive nitrogen groups present in the solute may interfere with the polymerisation reaction and prevent microcapsule formation.

Electron microscopy, permeability measurements and molecular weight cut off determinations suggested that the walls of the polyphthalamide microcapsules were morphologically complex and indicated the presence of pores in the membranes as previously reported by Chang and others (29). Studies on the <u>in vitro</u> release of low molecular weight solutes (molecular weight 200 to 500) from the microcapsules suggested that these solutes readily permeated the microcapsule walls and it is considered that the solutes diffused through the membranes via the pores. <u>In vivo</u> investigations also indicated that the microcapsules did not

significantly control the release of pilocarpine nitrate although the dwell time of the microcapsules in the eye was considerably greater than that obtained using aqueous solutions.

Attempts to modify or reduce the in vitro release rate characteristics of the microcapsules by modifications to the microcapsule core and wall were unsuccessful. Further investigation of the properties of these modified microcapsules is necessary in order to characterise them and to determine the effect of the modifications on pore size. Comparative determinations of pore size may be achieved by molecular weight cut off studies or by investigation of the relationship between molecular volume and rate of release of various solutes from different microcapsule preparations. In addition, film permeability measurements or the development of an in vitro model to simulate drug release in the eye would assist in predicting in vivo release profiles. Selection of the optimal method by which to reduce pore size and control release in vivo as a basis for further modifications may then be possible. Such modifications may include the preparation of multiple walled microcapsules, the use of different crosslinking agents or the preparation of microcapsules with walls formed by different polymers. An alternative approach to preparing polyamide microcapsules with an aqueous core and different release characteristics may be to freeze dry microcapsules containing an organic phase. This organic phase would then be replaced by an aqueous core by immersion of the dried capsules in water. Development of a system in which measurable sustained release is observed would then enable factors affecting the release from the microcapsules to be studied.

This project has served to indicate the feasibility of using polyphthalamide microcapsules for the sustained release of such drugs as pilocarpine nitrate to the eye. It has been shown that the dwell time of the microcapsules in the eye is significantly greater than that obtained using aqueous solutions although the microcapsule walls are freely permeable to pilocarpine nitrate and do not control its release. Considerable further work is necessary in order to modify the microcapsule preparation conditions to produce microcapsules with walls of a less porous nature which are effective in controlling the release of small solutes.

APPENDIX

A.1.1 Least Squares Linear Regression Analysis

When a linear relationship is assumed to exist between two variables, it is usual to fit a straight line by least-squares regression analysis. The simplest statistical model for this type of analysis assumes that the independent variable X is known without error of measurement, and that the corresponding measured values of the dependent variable Y are scattered normally about their true values. Hence each value Y_i of the dependent variable is normally distributed about a mean.

The method of least squares regression analysis obtains estimates of the intercept a, and the slope b, in the linear equation $Y = a + bX$ such that the sum of the squares of the deviations of the observation Y_i from their mean values $\alpha + \beta X_i$ is a minimum.

These values are

$$a = \frac{\Sigma Y_i - b\Sigma X_i}{n} \qquad \text{(equation A.1)}$$

$$= \bar{Y} - b\bar{X} \qquad \text{(equation A.2)}$$

$$b = \frac{n\,\Sigma X_i Y_i - \Sigma X_i \Sigma Y_i}{n\,\Sigma X_i^2 - (\Sigma X_i)^2} \qquad \text{(equation A.3)}$$

$$= \frac{\Sigma (X_i - \bar{X})\,(Y_i - \bar{Y})}{\Sigma (X_i - \bar{X})^2} \qquad \text{(equation A.4)}$$

where n is the number of points on the line

A.1.2 Correlation Coefficient

The correlation coefficient (r) is defined as:

$$r = \frac{\Sigma(X_i - \bar{X})\ (Y_i - \bar{Y})}{\sqrt{(X_i - \bar{X})^2\ (Y_i - \bar{Y})^2}} \qquad \text{(equation A.5)}$$

To represent a linear relationship between two variables X and Y, r must be ± unity. The calculated value of r is compared with the tabulated value at the 5% probability level for n-2 degrees of freedom, and if found to be greater than the tabulated value, the observations are considered to be linearly related.

A.1.3 Variance of the Slope

The variance of the slope (b) is termed S_b^2 and is given by the equation:

$$S_b^2 = \frac{\sigma e^2}{\Sigma(X_i - \bar{X})^2} \qquad \text{(equation A.6)}$$

where σe^2 is the residual variance of the dependent variable Y and is obtained from:

$$\sigma e^2 = \frac{\Sigma D^2}{n-2} \qquad \text{(equation A.7)}$$

where ΣD^2 is the residual sum of squares which is obtained from the equation:

$$\Sigma D^2 = (Y_i - \bar{Y})^2 - b^2\Sigma(X_i - \bar{X})^2 \qquad \text{(equation A.8)}$$

The standard deviation of the slope is given by the square root of the variance.

A.1.4 Variance of the Intercept

The variance of the intercept (a) is termed S_a^2 and is given by the equation:

$$S_a^2 = \frac{\Sigma X_i^2 - \sigma e^2}{n \Sigma (X_i - \bar{X}^2)} \qquad \text{(equation A.9)}$$

where $\sigma e^2 = \frac{\Sigma D^2}{n - 2}$

The standard deviation of the intercept is given by the square root of the variance.

A.1.5 To Determine the Equality of Two Estimates of a Parameter

This test is generally termed the Student 't' test. The equality of estimates P_1 and P_2 with variance S_1^2 and S_2^2 respectively of a parameter P is assessed by means of the following statistic:

$$t = \frac{P_1 - P_2}{\sqrt{S_1^2 + S_2^2}} \qquad \text{(equation A.10)}$$

The value of 't' is compared with tabulated values with $n_1 + n_2 - 4$ degrees of freedom where n_1 and n_2 are the number of observations used in the estimation of P_1 and P_2 respectively. If the calculated value of 't' exceeds the tabulated value at the 5% probability level, the parameters are considered to be significantly different at that level.

A.2 Determination of Pore Size Distribution from Nitrogen Adsorption Techniques

The pore size distribution from which the mean pore radius reported in Section 4.4 was calculated is given in Table A.1. This was calculated according to the work sheet in Table A.2. An explanation of this work sheet is given below.

Columns 1 and 2 in Table A.2 represent the data obtained directly from the desorption isotherm. The adsorbed volumes are normalised for one gram of adsorbent.

Table A.2

Work Sheet for Calculation of Pore Size Distribution

1	2	3	4	5	6	7	8	9	10	11	12	13	14
P/Po	Vgas STP	r_k	t	rp	$\bar{r}k$	$\bar{r}p$	Δt	ΔVgas STP	ΔVliq $\times 10^3$	$\Delta t\Sigma S$ $\times 10^3$	Vp $\times 10^3$	S	ΣS
	cm^3g^{-1}	A^o	A^o	A^o	A^o	A^o	19^o	cm^3g^{-1}	cm^3g^{-1}	cm^3g^{-1}	cm^3g^{-1}	m^2	m^2

Column 3 - the Kelvin radius (rk) is calculated from the Kelvin equation assuming a zero wetting angle. The Kelvin equation is given by:

$$rk = \frac{-2y\bar{V}}{RT \text{ in } P/Po} \qquad \text{(equation A.11)}$$

where P is the equilibrium vapour pressure of the liquid contained in a narrow pore of radius rk

Po is the equilibrium pressure of the same liquid exhibiting a plane surface

y is the liquids surface tension

$\bar{V}$ is the liquids molar volume

R and T are the gas constant and temperature respectively

TABLE A.1

Nitrogen Desorption Isotherm for Polyphthalamide Microcapsules

P/Po	r_k (A^o)	t (A^o)	rp (A^o)	$\bar{r}_k$ (A^o)	$\bar{r}p$ (A^o)	Δt (A^o)	ΔV_{gas} (cm^3)	$\Delta V_{kg} \times 10^3$ (cm^3)	$\Delta t \Sigma S \times 10^3$ (cm^3)	$V_p \times 10^3$ (cm^3)	S (m^2)	ΣS (m^2)
0.95	186	16.2	202	138.3	152.5	3.5	0.78	1.20	0	1.46	0.19	0.19
0.90	90.6	12.7	103	74.7	86.4	1.7	0.44	0.68	0.032	0.87	0.20	0.39
0.85	58.7	11.0	69.7	50.8	61.2	1.1	0.06	0.092	0.045	0.071	0.023	0.41
0.80	42.8	9.9	52.7	34.8	44.0	1.4	0.17	0.26	0.057	0.33	0.15	0.56
0.70	26.8	8.5	35.3	22.8	30.8	1.0	0.16	0.25	0.056	0.35	0.23	0.79
0.60	18.7	7.5	26.2	16.3	23.4	0.7	0.20	0.31	0.055	0.53	0.57	1.36
0.50	13.8	6.8	20.6	12.1	18.6	0.6	0.16	0.25	0.082	0.39	0.42	1.78
0.40	10.42	6.2	16.6	9.18	15.05	0.6	0.12	0.19	0.11	0.21	0.28	2.06
0.30	7.93	5.6	13.5									

If nitrogen is the adsorbate at its boiling point of $77^{o}K$, the working equation becomes

$$rk = \frac{4.15}{\log Po/p} \quad (A^{o}) \qquad \text{(equation A.12)}$$

Column 4 - the film depth t is calculated using the Halsey equation. For nitrogen this is written as

$$t = 3.54 \left[\frac{5}{2.303 \log Po/p}\right]^{1/3} \qquad \text{(equation A.13)}$$

where 3.54 is the estimated diameter in Angstroms of a nitrogen molecule in a close hexagonal packed liquid structure.

Column 5 is the pore radius (rp) calculated from

$$rp = rk + t \qquad \text{(equation A.14)}$$

Columns 6 and 7 $\bar{r}k$ and $\bar{r}p$ are prepared by calculating the mean value in each decrement from successive entries.

Column 8 the change in film depth Δt is calculated by taking the difference between successive values of it.

Column 9 $\Delta Vgas$ is the change in adsorbed volume between successive P/Po values and is determined by subtracting successive values from column 2.

Column 10 $\Delta Vliq$ is the volume of liquid corresponding to $\Delta Vgas$. The most direct way to convert $\Delta Vgas$ to $\Delta Vliq$ is to calculate the moles of gas and to multiply by the liquid molar volume. For nitrogen at standard temperature and pressure this is given by

$$\Delta Vliq = \frac{\Delta Vgas}{22.4 \times 10^{3}} \times 34.6 \qquad \text{(equation A.15)}$$

Column 11 represents the volume change of the adsorbed film remaining on the walls of the pores from which the centre core has previously evaporated. This volume is the product of the film area and the decrease in the film depth. It is necessary to assume no pores are larger than 950 A^{o} (P/Po = 0.99) and therefore the first entry in column 11 is zero. Subsequent entries are calculated as the product of Δt for a decrement and ΣS from the row above.

Column 12 the actual pore volume is calculated from the volume of liquid given in column 10. This volume is composed of the volume evaporated out of the centre cores plus the volume desorbed from the film left on the pore walls. Therefore since

$$\Delta Vliq = \pi \bar{r}_k^2 L + \Delta t \Sigma S \qquad \text{(equation A.16)}$$

where L is the pore length

and $Vp = \pi \bar{r}_p^2 L$

by combining the two previous equations the actual pore volume

$$Vp = \frac{\bar{r}_p}{\bar{r}_k}^2 [\Delta Vliq - (\Delta t \Sigma S)\ (10^{-4})] cm^3 \qquad \text{(equation A.17)}$$

Column 13 is the surface area of the pore walls calculated from the pore volume by

$$S = \frac{2Vp}{\bar{r}p} \times 10^4\ (m^2) \qquad \text{(equation A.18)}$$

where Vp is given in cm^3 and $\bar{r}p$ in A^{o}.

Column 14 is the summation of the value S multiplied by Δt from the following decrement to calculate the film volume decrease in column 11.

Summation of the values in column 12 leads to an estimation of the total volume of the pores ΣVp.

On the assumption that pores are cylindrical the average pore radius r_{av} is calculated using the equation

$$r_{av} = 2 \frac{\Sigma Vp}{\Sigma S} \qquad \text{(equation A.19)}$$

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